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Effect of nitrogen fertilizer rates on grain yield and nitrogen uptake and use efficiency of bread wheat (*Triticum aestivum* L.) varieties on the *Vertisols* of central highlands of Ethiopia

Fresew Belete^{1*} , Nigussie Dechassa¹, Adamu Molla² and Tamado Tana¹

Abstract

Background: Nitrogen is one of the most important nutrients limiting yield of bread wheat in Ethiopian highlands. Application of a large amount of N fertilizer has been a method of increasing yield in the study area which is costly and can cause environmental pollution. Therefore, a field experiment was conducted for two consecutive years (2014 and 2015) to evaluate the effect of N application rates on grain yield, nitrogen uptake and N use efficiency of bread wheat varieties. The treatments consisted of a factorial combination of four rates of nitrogen (0, 120, 240 and 360 kg ha⁻¹) and three popular wheat varieties (Menze, Tsehay and ET-13) in a randomized complete block design with four replications.

Results: The two-way interaction of year and N rate, N rate and variety significantly affected grain nitrogen concentration. The interaction of year and variety and N rate and variety affected grain and straw nitrogen concentrations, respectively. The three-way interaction of year, N fertilizer rate and variety significantly affected ($P < 0.01$) grain yield, grain and total nitrogen uptake, agronomic efficiency, agro-physiological efficiency and apparent recovery efficiency. The highest grain yield (5718.32 kg ha⁻¹) was obtained from variety Menze at N rate of 360 kg ha⁻¹ in 2015 which was statistically at par with the application of 240 kg N ha⁻¹ and with variety Tsehay at N rate of 240 and 360 kg N ha⁻¹ during the same growing season. The highest grain and straw (2.7 and 0.35%, respectively) nitrogen concentration were produced by variety Tsehay at N rate of 360 kg ha⁻¹. The highest apparent nitrogen recovery efficiency was recorded for variety Tsehay at N rate of 120 kg ha⁻¹ in both growing seasons.

Conclusion: Hence, it could be concluded that varieties Menze and Tsehay produced the highest grain yield followed by variety ET-13. All wheat varieties tested did not show a significant variation in most of the nitrogen use efficiency parameters, and these efficiency parameters decreased with the increasing rate of N beyond 120 kg ha⁻¹ in both growing seasons.

Keywords: Concentration, Grain, Recovery, Uptake, Yield

*Correspondence: beletefresew@gmail.com

¹ School of Plant Sciences, College of Agriculture and Environmental Sciences, Haramaya University, P.O. Box 138, Dire Dawa, Ethiopia
Full list of author information is available at the end of the article



Background

Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops of the world and is a staple food for about one third of the world's population [1]. It is one of the major cereal crops grown in the highlands of Ethiopia, and the country is regarded as the largest wheat producer in Sub-Saharan Africa [2]. Out of the total grain crop area, wheat ranked 4th after *tef* (*Eragrostis tef*), maize (*Zea mays*) and sorghum (*Sorghum bicolor*), while third in total production after maize and *tef* [3]. Despite the long history of wheat cultivation and its importance to the Ethiopian agriculture, its average yield is still very low, not exceeding 2.4 t ha⁻¹ [4] which is below the world's average of 3.4 t ha⁻¹ [5]. The low yield of wheat in the country may be due to the use of low-yielding varieties, inadequate and erratic rainfall, diseases, low soil fertility and waterlogging in the *Vertisol* areas [6].

Vertisols occupy about 12.6 million hectares in the country where 7.6 million ha was found in the highlands. These soils are characterized by very low water infiltration rate or low saturated hydraulic conductivity and, therefore, are susceptible to waterlogging under high-intensity rainfall conditions. In addition, the Ethiopian *Vertisols* tend to exhibit low total N content mainly due to leaching and denitrification [7] limiting wheat production; hence, application of N is one of the major inputs used by farmers to achieve desired crop yields on these soils [8]. However, nitrogen use efficiency (NUE) in cereals is generally poor, where it is estimated that only 30–40% of the total of N-fertilizers applied are actually harvested in the grain. The remainder of the applied N is lost to the soil through leaching and runoff, where the often excessive application can affect natural ecosystems through N pollution. Loss of N also contributes to significant direct economic losses to the grower particularly when N fertilizer costs are high [9].

Thus, the application of the appropriate rate of N fertilizer is considered to be a primary means of increasing wheat grain yield, in improving N uptake and use efficiency and consequently nitrogen harvest index [10]. Many studies have shown genetic variation of wheat in nitrogen utilization efficiency (NUE) and nitrogen uptake efficiency (NUpE) and nitrogen recovery efficiency (NRE) [11] and that the use of the best-adapted genotype can contribute to improved efficiency in how cereal crops acquire and use soil N or fertilizer N. The studies showed that interactions of genotype and nitrogen supply affect NUE. However, improved varieties are often developed without considering their ability to grow and yield under low soil nutrient status and have been selected for high yield under high nutrient input conditions [12]. In contrast, the use of modern wheat cultivars can contribute to improve nitrogen use efficiency [13]. In

addition, studies indicate that the development and use of wheat cultivars with higher NUE can contribute to reducing the amount of nitrogen to be applied without decreasing grain yield [14]. Therefore, selection of wheat varieties with high NUE on the basis of agricultural practices could increase economic return and reduce environmental pollution.

In Ethiopia, wheat is grown during high rainfall season and loss of applied N through leaching, denitrification and runoff especially in *Vertisols* is the major factors which resulted in inefficient use of N fertilizer [15]. This forced farmers in the study area to use a large amount of N fertilizer (256 kg N ha⁻¹) as a compensation for the lost N which is beyond the recommended rate of 87 kg N ha⁻¹ [16]. Such overuse of nitrogenous fertilizers can be a major source of environmental pollution (ground water pollution), due to nitrate leaching and runoff [17], and is costly. These drawbacks have motivated in making important changes in recent fertilization practices such as use of reduced amount of nitrogen fertilizer in combination with wheat varieties with elevated nitrogen use efficiency. However, limited research has been done on the effects of N rate in relation to genetic variations for NUE in Ethiopia. Therefore, the present study was conducted to evaluate the effect of nitrogen application on grain yield, nitrogen uptake and nitrogen use efficiency of wheat varieties at Enewari, central highlands of Ethiopia.

Methods

Description of the study site

The study was conducted for two consecutive years from July to December during the 2014 and 2015 main cropping seasons in the district of Moretina-Jiru at the Enewari experimental field station in the central highlands of Ethiopia. Enewari is located at 9°52'N latitude and 39°10'E longitude at an altitude of 2680 meters above sea level. This area is typical of the rain-fed wheat-growing regions of Ethiopia with average annual rainfall of 1153.69 mm. The dominant soil type of the area is *Vertisol* known for its high waterlogging and drainage problems.

Rainfall in the study area varied considerably between the two growing years. The total rainfall during the cropping season (July–December) was 826.8 mm and 545.6 mm in 2014 and 2015, respectively. Differences in temperatures between the two growing seasons were relatively modest (Fig. 1). Prior to planting, surface (0–20 cm) soil samples, from ten spots across the experimental field, were collected, composited and analyzed for soil physicochemical properties at Debre Berhan Agricultural Research laboratory and results are presented in Table 1.

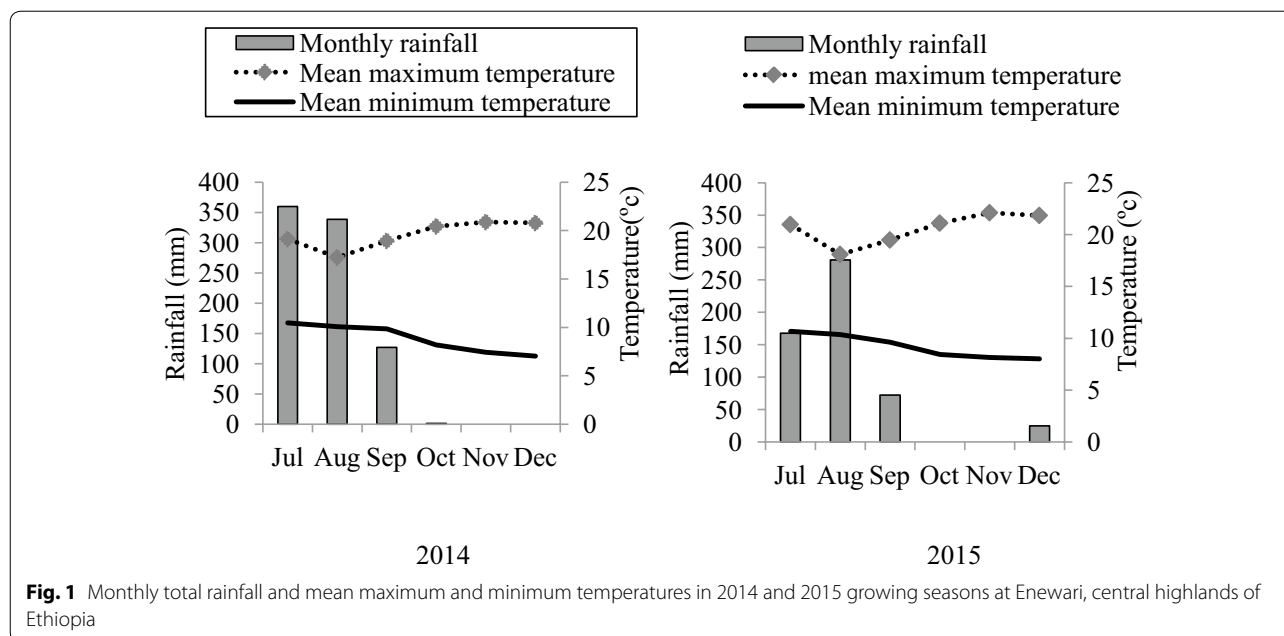


Fig. 1 Monthly total rainfall and mean maximum and minimum temperatures in 2014 and 2015 growing seasons at Enewari, central highlands of Ethiopia

Table 1 Soil physicochemical properties at depth of 0–20 cm during the years of 2014 and 2015 before sowing of bread wheat

Parameter	Values		Rating	References
	2014	2015		
Sand (%)	11.25	8.75	–	–
Silt (%)	22.5	22.5	–	–
Clay (%)	66.25	68.75	–	–
Texture lass	Clay	Clay	–	–
pH 1:2.5 (H ₂ O)	6.92	7	Neutral	Tekalign [18]
Organic carbon (%)	0.96	1.1	Low	Tekalign [18]
Total N (%)	0.05	0.06	Low	Tekalign [18]
Avail. P (ppm)	6.44	7.72	Low	Olsen et al. [19]

Description of the experimental materials

Three popular bread wheat varieties, namely, Menze, Tsehay and ET-13, were used as a test crop. ET-13 is a tall variety, while Tsehay and Menzie are medium in height. All of them are recommended for the area as they are high yielding and resistant to yellow rust. Fertilizers used for the study were N in the form of urea [CO (NH₂)₂] (46% N) and phosphorus in the form of triple superphosphate (TSP) (46% P₂O₅) (Table 2).

Treatments and experimental design

The treatments consisted of factorial combinations of four N fertilizer rates (0, 120, 240 and 360 kg ha⁻¹) and three wheat varieties (Menzie, Tsehay and ET-13) laid out

Table 2 Description of the wheat varieties used in the study area *Source:* Crop variety register issue no. 12 (2009) and Wheat Technology Implementation Manual (2014)

No.	Descriptor	Variety		
		Menzie	Tsehay	ET 13
1	Year of release	2007	2011	1981
2	Stature	64 cm	76 cm	–
3	Number of days to maturity	154	138	127–149
4	Grain color	White	Medium white	–

in a randomized complete block design (RCBD) with four replications. The plot size for planting was 1.6 m × 3.0 m (4.8 m²) accommodating 8 rows spaced 20 cm apart. Four central rows with a net plot size of 2.4 m² were used for data collection and measurement. The distance between the plots and blocks were kept at 0.5 m and 1 m apart, respectively.

Experimental procedures

The experimental plots were prepared by tractor plowing and harrowing. In accordance with the specifications of the design, a field layout was prepared and each treatment was assigned randomly to experimental plots within each block independently. Wheat seed was sown by drilling in rows at the recommended rate of 150 kg ha⁻¹ on July 24th in both years. Each year, all the wheat plots were supplied with triple superphosphate

(TSP) at a recommended rate of 138 kg P₂O₅ ha⁻¹ once at sowing [16], whereas nitrogen was applied in the form of urea in split, i.e., half at sowing and the remaining half at tillering. Plots were kept free of weeds by hand weeding. No insecticide or fungicide was applied since there was no outbreak of insects or diseases. Harvesting was done manually using hand sickle.

Plant sampling and analysis

At crop maturity, a subsample from each net plot was harvested at ground level and dried at 70 °C until constant weight was reached for dry weight determination and partitioned into straw and grain. The dried samples were milled, and the grain and straw N content of the plant samples were determined using the micro-Kjeldahl method as stated by American Association of Cereal Chemists (AACC) [20]. The laboratory analysis was done at Soil Laboratory Department of the Debre Berhan Agricultural Research Center.

Data collected

The grain yield (kg ha⁻¹) was determined after threshing the sun-dried plants harvested from each net plot area, and the yield was adjusted at 12.5% moisture content. Total grain N uptake (Ng) in kg ha⁻¹ was calculated by multiplying total grain yields by their respective N content percentages. Total nitrogen uptake (TNU) was calculated as the sum of the respective Ng and straw nitrogen uptake values. Then, nitrogen recovery and nitrogen use efficiency by the crop were determined using the formulae described by Fageria et al. [21]:

Agronomic efficiency (kg kg⁻¹) It is defined as the economic production obtained per unit of nitrogen applied and was calculated as: $AE \text{ (kg kg}^{-1}\text{)} = \frac{Gf(\text{kg}) - Gu(\text{kg})}{Na(\text{kg})}$ where

AE stands for agronomic efficiency, Gf and Gu for grain yield in fertilized and unfertilized plots, respectively, and Na for quantity of fertilizer applied.

Apparent fertilizer N recovery efficiency (%) It indicates the quantity of nutrient uptake per unit of nutrient applied and was calculated as: $ARE \text{ (\%)} = \frac{Nf - Nu}{Na} \times 100$ where Nf is the total N uptake of the fertilized plot (kg), Nu is the total N uptake of unfertilized plot (kg) and Na is the quantity of N applied (kg).

Agro-physiological efficiency (APE) (kg kg⁻¹) was determined as $\frac{Gf(\text{kg}) - Gu(\text{kg})}{Nf(\text{kg}) - Nu(\text{kg})}$ where Gf and Gu stand for grain yield in fertilized and unfertilized plots, respectively, Nf is the total N uptake of the fertilized plot (kg) and Nu is the total N uptake of unfertilized plot (kg).

Finally, the nitrogen harvest index (NHI) was determined as the ratio of nitrogen uptake by grain and nitrogen uptake by grain plus straw as described by Fageria et al. [10].

Data analysis

After verifying the homogeneity of error variances, combined analysis of variance was done using the procedure of SAS [22]. Mean comparisons were done by Duncan's multiple range test [23] at the 5% level of significance.

Results and discussion

Grain yield

The analysis of variance for grain yield showed significant difference between years, and among nitrogen rates and varieties. The interaction of year × nitrogen, year × variety, N rate × variety and year × N rate × variety was also significant (Table 3). The highest grain yield (5718.32 kg ha⁻¹) was obtained for variety Menze at 360 kg N ha⁻¹ in 2015, which was statistically similar with the grain yield obtained at N rate of 240 kg N ha⁻¹ and with variety Tsehay at a rate of 240 and 360 kg N ha⁻¹ at the same growing season, and the lowest grain yield was recorded in three of the varieties with no nitrogen application in both years (Table 4). This implies genotypes differ in absorption, and utilization of N depends on the environment. Grain yield is the function of genotype, environment and genotype–environment interaction [24].

In addition, the application of nitrogen increased drastically grain yield of the varieties tested as compared to the control in both growing years. The increase in yield of the varieties with increasing N rates up to adequate level might be due to the role of N in increasing the leaf area and promote photosynthesis efficiency which promote dry matter production and increase yield. In line with this, improvements in wheat yield and its components under the acceptable increasing N rates were reported by Stickse et al. [25].

In this study, in contrast to varieties Menze and Tsehay, N application beyond 240 kg N ha⁻¹ led to a reduction in grain yield of variety ET-13 in 2015, due to the increase in lodging. This might be attributed to the tall growth nature of the variety and the favorable amount rainfall during 2015 which increased N uptake efficiency and induced excessive vegetative growth at the highest N rate, and therefore, early lodging of ET-13 was observed. This result is in line with [26], who reported that excess application of N resulted in lodging of wheat and caused a dramatic yield decrease. Furthermore, Ercoli et al. [27] compared old and modern varieties under different N soil levels, where they found that modern varieties produced higher yields than the old varieties in both N-poor and N-rich environments as they can exploit the soil N effectively.

The present study further revealed that in 2014 (the year characterized by a high amount of rainfall) Menze and Tsehay varieties with N inputs greater than

Table 3 Mean squares of combined analysis of variance for the effects of year, N rate and variety on grain yield, grain nitrogen concentration (GNC), straw nitrogen concentration (SNC), grain nitrogen uptake (GNUP), total nitrogen uptake (TNUP), agronomic efficiency (AE), agro-physiological efficiency (APE), recovery efficiency (RE) and nitrogen harvest index (NHI) at Enewari, central highlands of Ethiopia

Parameter	Mean squares for sources of variation with respective degrees of freedom in parenthesis								
	Y (1)	Rep (Y) (6)	N (3)	V (2)	N×V (6)	Y×N (3)	Y×V (2)	Y×N×V (6)	Error (66)
GY	1,062,684**	23,718	64,523,847**	6,214,722**	1,240,489**	188,153**	1,120,317**	624,803**	39,103**
GNC	0.16*	0.013	2.38**	0.53**	0.07**	0.04*	0.31**	0.01NS	0.01
SNC	0.021**	0.001	0.143**	0.031**	0.012**	0.001NS	0.003NS	0.003NS	0.001
GNUP	50.63 ns	22.36	41,509.00**	5241.11**	858.91**	173.17**	26.39 ns	391.82**	27.6
TNUP	1611.76**	19.44	69,011.00**	4385.22**	1018.49**	456.28**	19.75NS	396.92**	38.12
AE	15.38*	1.53	2531.80**	39.82**	10.44**	5.64**	25.12**	4.39**	1.03
APE	33.12*	6.48	7744.88**	102.85**	36.68**	17.59**	111.22**	13.56**	3.60
RE	0.024**	0.00042	1.53**	0.069**	0.01**	0.0078**	0.0018NS	0.0036**	0.0008
NHI	0.0655NS	0.0014	0.0299*	0.0523**	0.0045**	0.0002 ns	0.0002 ns	0.0029 ns	0.0012

Y year; N nitrogen rate; V variety

*Significant at the 0.05 probability level; **Significant at $P < 0.01$ probability level**Table 4 Interaction effect of year, N rate and variety on grain yield (kg ha^{-1}) of bread wheat at Enewari, central highlands of Ethiopia**

Variety	N rate (kg ha^{-1})	Year 2014	Year 2015
Menzie	0	1498.44i	1420.51i
	120	4218.79fg	4553.19d-g
	240	4781.44c-e	5457.60ab
	360	5012.44b-d	5718.32a
Tsehay	0	1258.13i	1367.41i
	120	4196.92fg	4532.61d-g
	240	4644.28d-f	5234.55a-c
	360	4798.73c-e	5535.09ab
ET-13	0	1100.13i	1292.17i
	120	4003.56g	4210.28fg
	240	4123.73fg	4120.57fg
	360	4255.91e-g	2975.27h
CV (%)		5.3	

Means followed by the same letter(s) for the same parameters are not significantly different from each other at 5% level of significance according to Duncan's multiple range test

120 kg ha^{-1} suffered a greater yield reduction as compared to the year 2015. However, variety ET-13 had increased yield with the highest N rate in 2014 as compared to 2015 (Table 4). In general, varieties Menze and Tsehay were high yielders as compared to the variety ET-13 in both growing years. But, variety ET-13 was stable in yield in both growing years at N rate of 120 and 240 kg ha^{-1} , whereas varieties Menze and Tsehay produced significantly higher grain yield in 2015 as compared to 2014 for these two N rates. This implies that the two recent varieties were less resistant to waterlogging

which occurred in 2014 as compared to 2015. High rainfall in 2014 might have aggravated the waterlogging effect which causes either incomplete remobilization of nitrogen or a reduction in the amount of nitrogen available for remobilization [28]. Waterlogging due to higher rainfall resulted in the leaching of nitrate below the rooting depth of crops and denitrification, a major loss of N fertilizer that can reduce grain yield. Similarly, there is a strong interaction between waterlogging and crop fertilizer response, with an inefficient use of available N in years characterized by high rainfall [29]. A lower N uptake in wheat crop resulted in a decrease in grain yield [28].

Nitrogen concentration

Straw nitrogen concentration

Nitrogen concentration in straw was significantly affected by the main effects of year, N rate and variety as well as by the interaction of N rate and variety. However, the interaction of year \times N rate, year \times variety and year \times N rate \times variety was nonsignificant (Table 3). The highest amount of straw nitrogen concentration (0.35%) was recorded from variety Tsehay at N rate of 360 kg ha^{-1} which was statistically at par with the application of 240 kg N ha^{-1} and with variety Menze at N rate of 240 and 360 kg ha^{-1} . However, the lowest nitrogen concentration in straw (0.11%) was obtained from variety Tsehay under the control treatment and it was statistically at par with varieties Menze and ET-13 (Table 5). In general, straw nitrogen concentration of the tested varieties increased as amount of nitrogen increased from 0 to 360 kg N ha^{-1} except for variety ET-13.

The higher N concentration of straw with the increase in N rate might be due to the availability of sufficient

Table 5 Interaction effect of N rate and variety on nitrogen concentration of straw and grain of bread wheat at Enewari, central highland of Ethiopia

Variety	Straw nitrogen concentration (%)			
	N rate (kg ha ⁻¹)			
	0	120	240	360
Menze	0.14ef	0.28bc	0.30ab	0.34a
Tsehay	0.11f	0.23cd	0.32ab	0.35a
ET-13	0.16ef	0.18de	0.26bc	0.23cd
CV (%)	15.03			
Variety	Grain nitrogen concentration (%)			
Menze	1.89f	1.8f	2.17de	2.3cd
Tsehay	1.86f	2.06e	2.43bc	2.7a
ET-13	1.75f	1.78f	2.17de	2.48b
CV (%)	4.49			

Means followed by the same letter are not significantly different from each other at 5% level of significance according to Duncan's multiple range test

nitrogen for the crop which might increase N concentration in the biological yield especially straw because of healthier roots and greater density. The increase in straw N concentration observed with increasing N input is in agreement with the results of Worku et al. [30] who found an increase of 40% N content in wheat straw with the increasing N level from 0 to 210 kg ha⁻¹ for wheat crop. Similarly, straw nitrogen of bread wheat varieties exhibited a progressive increase in response to the increasing levels of N from 0 to 120 kg ha⁻¹ [31]. The result also showed that Tsehay and Menze varieties proved to contain the highest N concentration in their straw at the highest N rate compared with variety ET-13. This could be due to a better genetic response of Menze and Tsehay varieties to applied N.

Grain nitrogen concentration

Nitrogen concentration in the grain was significantly affected by year, N rates, varieties and the interaction of N rate × variety, year × N rate and year × variety. But, the three-way interaction effect of year, N rate and variety for grain N concentration was not significant (Table 3). As to the interaction effect of N rate × variety, the highest amount of nitrogen concentration in the grain (2.7%) was recorded from variety Tsehay at N rate of 360 kg ha⁻¹. However, the lowest amount of nitrogen concentration in the grain (1.75%) was recorded from variety ET-13 from the control treatment which was statistically at par with varieties Menze and ET-13 under the N rate of 120 kg ha⁻¹ and the control treatment (Table 5). The highest grain nitrogen concentration in variety Tsehay at the highest N rate might be due to its better

Table 6 Interaction effect of year and N rate, year and variety on grain nitrogen concentration (%) of bread wheat at Enewari, central highlands of Ethiopia

N rate (kg ha ⁻¹)	Year 2014	Year 2015
0	1.90cd	1.77e
120	1.96c	1.80de
240	2.25b	2.27b
360	2.51a	2.47a
Variety		
Menze	2.12b	1.96c
Tsehay	2.38a	2.15b
ET-13	1.97c	2.12b
CV (%)	4.49	

Means followed by the same letter are not significantly different from each other at 5% level of significance according to Duncan's multiple range test

genetic response to the applied N and translocation of the absorbed N to the grain. Similarly, Lopez-Bellido et al. [32] reported that genotypic variability in grain N concentration may be affected not only by physiological traits but also by N supply in the soil.

In Menze, Tsehay and ET-13 varieties, grain nitrogen concentration increased with increasing N level showing the highest values always with the highest N level (360 kg ha⁻¹). The increase in grain nitrogen concentration of the varieties at highest N rate might be due to the sufficient availability of nitrogen that increased N mobilization to the grain at grain filling stage. Similarly, increasing N rates from 60 to 120 kg ha⁻¹ resulted in the increase in grain nitrogen concentration from 1.52 to 2.28% in bread wheat [33]. The result further revealed that variety Menze produced the lowest grain nitrogen concentration at a rate of 120 kg ha⁻¹ which was lower than the control (without N) in contrast to variety Tsehay and variety ET-13, and this could be due to dilution and N mobility to the vegetative part. In agreement with this result, Sinebo et al. [34] reported that grain N content showed at first decreasing trend with increasing yield (increasing N supply) before both yield and grain N concentration increased with increasing N supply.

With regard to year × N rate, the highest grain nitrogen concentration was recorded with the application of 360 kg N ha⁻¹ in both growing years, while the lowest grain nitrogen concentration was recorded from the control treatment which was statistically similar with the grain nitrogen content obtained at N rate of 120 kg ha⁻¹ in 2015 (Table 6). As for N level, grain nitrogen content increased with increasing N level in both growing years showing the highest values always with the application of the highest N rate (360 kg N ha⁻¹) (Table 6). There was a significant decline in 2015 as compared with 2014 at rates of 120 kg N ha⁻¹ and the control treatment where

there was no significant difference in grain nitrogen concentration between years with the application of 240 and 360 kg N ha⁻¹. The lower rainfall condition in 2015 reduced the problem of waterlogging and increased the availability of N which increased the aboveground biomass production under the lower N rates and reduced the amount of nitrogen translocated to the grain (dilution effect), whereas increasing the level of nitrogen beyond 240 kg ha⁻¹ did not significantly affect grain nitrogen concentration between the two growing seasons. This indicates that the application of N above 240 kg ha⁻¹ in both growing years had an effect on accumulation of nitrogen on the grain, resulted in both the increment of aboveground biomass and nitrogen accumulation in the grain.

As to the interaction effect of year × variety, variety Tsehay produced the highest amount of grain nitrogen concentration (2.38%) in the year 2014, while the lowest grain nitrogen concentration was produced by varieties ET-13 and Menze in the year 2014 and 2015, respectively (Table 6). In general, as compared to the year 2015 grain nitrogen concentration of variety Menze and variety Tsehay increased in 2014 in contrast to variety ET-13. The increment was 8.2% and 10.7% over that of 2015 for varieties Menze and Tsehay, respectively. The lower grain nitrogen concentration in the grain in the year 2015 for these varieties might be due to the increase in the aboveground biomass production and less nitrogen translocated to the grain. On the other hand, high rainfall in 2014 aggravated the problem of waterlogging which significantly reduced the availability of nitrogen and depressed aboveground biomass production and the varieties tended to accumulate a high amount of nitrogen to the grain as compared to the second growing season. In contrast to the two varieties, variety ET-13 produced significantly higher amount of grain nitrogen concentration in 2015 than 2014. Similar report was found that waterlogging increased the nitrogen concentration of the grain and straw mainly due to the reduction in dry weight. At the same soil and N fertilization level, the N concentration of the wheat plant parts declines with an increase in biomass possibly due to dilution effect [35].

Effect on nitrogen uptake

Grain nitrogen uptake

Grain nitrogen uptake reflected grain yield response to applied nitrogen fertilizer rate. The nitrogen uptake by the grain was significantly ($P < 0.01$) affected by the interaction of year, N rate and variety (Table 3). The highest amount of nitrogen uptake by the grain (143.13 kg ha⁻¹) was recorded from variety Tsehay at a rate of 360 kg N ha⁻¹ in 2015 which was statistically at par with the year 2014 for the same treatment and with Menze variety at N rate of 360 kg N ha⁻¹ in the year

2015. However, the lowest nitrogen uptake by grain (19.3 kg N ha⁻¹) was recorded for ET-13 variety and it was statistically at par with Menze and Tsehay varieties all at zero nitrogen fertilization (Table 7).

The result indicated that variety Tsehay had the highest amount of nitrogen uptake by the grain in both growing seasons which was statistically similar with variety Menze at the highest N rate in 2015. This shows that varieties Tsehay and Menze are more stable in providing higher yield and less sensitive to rainfall amount fluctuation observed in the 2 years under higher N rates. High rainfall in 2014 caused waterlogging and might have reduced availability of nitrogen for ET-13 variety, and the variety is also a low yielder which had a respective lower nitrogen uptake by the grain at the rates of 240 and 360 kg N ha⁻¹ (Table 7). However, this gap was narrowed down in 2015 for Menze variety due to the relatively lower rainfall amount which reduced the problem of waterlogging and runoff loss of the applied N which consequently increased the availability of nitrogen and raised the grain yield and grain nitrogen uptake under the highest N rate. In addition, the highest nitrogen uptake by the grain in both growing seasons by varieties Tsehay and Menze at the highest N rate might be due to the sufficient availability of nitrogen for the crop. However, variety ET-13 had abnormally low uptake of nitrogen by the grain at 360 kg N ha⁻¹ in the year 2015, matching the generally low grain yield of the variety at this N rate. This was mainly due to the lodging of variety ET-13 at the

Table 7 Interaction effect of year, N application rate and variety on grain nitrogen uptake and total nitrogen uptake of bread wheat at Enewari, central highland of Ethiopia

Variety	N rate (kg ha ⁻¹)	GNUP (kg ha ⁻¹)		TNUP (kg ha ⁻¹)	
		Year 2014	Year 2015	Year 2014	Year 2015
Menze	0	29.58j	25.42j	31.68k	28.86k
	120	81.42g-i	75.83hi	97.46ij	98.90ij
	240	106.87ef	114.90de	124.00gh	144.33c-f
	360	116.43c-e	130.42a-c	147.31c-e	165.30ab
Tsehay	0	24.65j	23.99j	26.70k	27.60k
	120	93.78fg	85.35gh	106.89hi	108.98hi
	240	116.17c-e	124.03b-d	139.15d-g	154.22b-d
ET-13	360	134.70ab	143.13a	160.72bc	178.50a
	0	19.30j	22.61j	23.68k	29.78k
	120	68.52i	77.54hi	86.21j	99.11ij
	240	83.15g-i	95.68fg	107.44hi	135.02e-g
	360	102.63ef	75.71hi	128.96fg	107.94hi
CV (%)		6.39		6.02	

Means followed by the same letter(s) for the same parameter are not significantly different from each other at 5% level of significance according to Duncan's multiple range test

highest N rate of 360 kg ha⁻¹ during this growing period which reduced the grain yield. A reduction in grain yield reduced the nitrogen uptake by the grain.

In general, variety Tsehay had the highest grain nitrogen uptake value, while variety ET-13 produced the least in both growing years under the different N rates. This is in confirmation with Yesuf and Duga [36] who reported significant increases in grain nitrogen uptake with increased N rate and also genetic differences for the trait.

Total nitrogen uptake

The total N uptake also reflected the biomass yield response of varieties to the applied N fertilizer rates. Total uptake of nitrogen differed significantly between year, N fertilizer rate and variety. Likewise, the interaction of year × N rate, year × variety and year × N rate × variety revealed a significant effect on total nitrogen uptake (Table 3). The highest total nitrogen uptake (178.5 kg ha⁻¹) was recorded for variety Tsehay at 360 kg N ha⁻¹ in 2015, with no significant difference with variety Menze at this rate in the same growing season, while the lowest total nitrogen uptake value was recorded from Menze, Tsehay and ET-13 varieties with no N application in both growing years (Table 7).

In general, varieties Tsehay and Menze exhibited a strongly positive response to total N uptake with the increase in N supply, where the highest values were recorded at N rate of 360 kg ha⁻¹. The increase in total N uptake at the highest N rate could be explained by the highest N within the grain of the varieties which allowed it to concentrate nitrogen as their yield increased. Similarly, Motzo et al. [37] reported that the highest grain N uptake of wheat resulted from the highest N rate (180 kg N ha⁻¹).

Correspondingly, the total nitrogen uptake was superior for varieties Menze and Tsehay under the highest N rate relative to variety ET-13 in both growing years where their yields were higher. This could be explained by the stable grain nitrogen uptake of the varieties, which allowed it to concentrate nitrogen as their yield increased. The lower N uptake value of variety ET-13 at the highest N rate, while possessing a higher grain N concentration in the year 2015, could be due to the lodging of the variety in the early reproductive phase which reduced its yield and indicates an inability of this old tall variety (ET-13) to use N when it is available at higher levels. This result was in agreement with the study by Amsal and Tanner [38] who reported higher total nitrogen uptake for a relatively high-yielding semi-dwarf variety under the highest N rate. In contrast, Moll et al. [39] reported no relationship between N uptake and year of release of the variety or plant height. But, when N is a limiting factor (under the control treatment), the ability

of ET-13 to explore the soil and absorb available N did not differ from that of the other two varieties. In general, most of the variation in total N uptake was due to differences in growth (total dry matter production) rather than to differences in N concentration (total N %). The varietal range of total nitrogen uptake under different N rates in this study was in the range of 23.68 and 178.5 kg N ha⁻¹ higher than previously reported in Ethiopia which ranged from 38.2 to 153 kg N ha⁻¹ for *Vertisol* sites [40] and from 65.4 to 112 kg N ha⁻¹ for non-*Vertisol* sites [38, 40] possibly due to the high rates of nitrogen applied in this study. In addition, total N uptake was higher in 2015 than 2014 under all N rates for varieties Tsehay and Menze. The higher N uptake in the year 2015 might be due to the lower rainfall condition which reduced the incidence of waterlogging and increased availability of nitrogen for the plants to grow and provide yield better than the year 2014. The difference recorded among the three wheat varieties in relation to total N uptake was in agreement with the results reported by Asnakew et al. [35].

Nitrogen use efficiency parameters

Agronomic efficiency

The analysis of variance indicated that the main effect of year, N rate, variety had a highly significant effect on agronomic efficiency (AE). Likewise, the interaction of year × N rate, year × variety, N rate × variety and year × N rate × variety revealed significant effect on agronomic efficiency (Table 3). The highest agronomic efficiency (26.38 kg kg⁻¹) was obtained from variety Tsehay at N rate of 120 kg ha⁻¹ in 2015. This result was statistically at par with variety Menze and variety ET-13 where increasing one unit of nitrogen improved grain yield by 26.11 kg and 24.32 kg grains per kg N⁻¹ applied, respectively, during the same growing season. However, the lowest agronomic efficiency value of 4.68 kg kg⁻¹ was recorded from variety ET-13 in 2015 when supplied with 360 kg N ha⁻¹ (Table 8). The agronomic efficiency of applied N decreased with the increasing levels of N for all the varieties in both growing years. A decreasing trend in nitrogen agronomic efficiency was reported with increasing N levels from 30 to 120 kg ha⁻¹ [33].

The result further indicated that, in 2015, the application of 120 kg N ha⁻¹ significantly increased agronomic efficiency of variety Menze when compared with the year 2014, while the application of N did not significantly affect agronomic efficiency of variety Tsehay and ET-13 at these nitrogen rates between the study years. This indicates that the higher rainfall in the year 2014 causes the problem of waterlogging which reduced availability of N for variety Menze, and this variety is sensitive to waterlogging and in absorbing N at lower N level as compared to the two varieties. Moreover, the agronomic efficiency

Table 8 Interaction effect of year, N application rate and variety on agronomic efficiency (AE), of bread wheat at Enewari, central highland of Ethiopia

Variety	N rate (kg ha ⁻¹)	AE (kg kg ⁻¹)	
		Year 2014	Year 2015
Menze	120	22.67b	26.11a
	240	13.68de	16.82c
	360	9.76fg	11.94ef
Tsehay	120	24.49ab	26.38a
	240	14.11cde	16.11cd
	360	9.84fg	11.58efg
ET-13	120	24.20ab	24.32ab
	240	12.60ef	11.79ef
	360	8.77g	4.68h
CV (%)		8.39	

Means followed by the same letter(s) are not significantly different from each other at 5% level of significance according to Duncan's multiple range test

of the varieties under the lower N rate (120 kg ha⁻¹) was not significantly different in 2015. The nonsignificant varietal difference in agronomic efficiency in the study at a rate of 120 kg N ha⁻¹ under the favorable environmental condition (2015) was in agreement with the findings of Dobermann [41] who reported less genetic variations and suggested that the trait would not be a breeding goal to modify. In contrast, Noureldin et al. [42] reported significant genetic variations among wheat varieties for N uptake efficiency.

Agro-physiological efficiency

Agro-physiological efficiency represents the ability of a plant to transform N acquired from fertilizer into economic yield (grain) [43]. Agro-physiological efficiency (APE) also varied significantly according to year and treatment (N fertilizer rates and variety), and all two- and three-way interactions were also significant (Table 3). The maximum APE (46.56 kg kg⁻¹) was observed for variety ET-13 at N rate of 120 kg ha⁻¹ in 2014, which was statistically at par with variety Menze and variety ET-13 at the same level of nitrogen during both growing years. This trait behaved in a similar manner to agronomic efficiency, i.e., there was low APE at high N levels for all the varieties tested, where APE reached 21.56 kg kg⁻¹ with the addition of 360 kg N ha⁻¹ for variety ET-13 during 2015 cropping period (Table 9). The difference among the varieties in APE obtained in this study was in agreement with the study by Gauer et al. [43] who reported that agro-physiological efficiency depends on genotypes. The maximum and minimum values of this parameter were recorded at the lowest and highest N rates, respectively,

Table 9 Interaction effect of year, N rate and variety on agro-physiological efficiency (APE) and apparent recovery efficiency (ARE) of bread wheat at Enewari, central highland of Ethiopia

Variety	N rate (kg ha ⁻¹)	APE (kg kg ⁻¹)		ARE (%)	
		Year 2014	Year 2015	Year 2014	Year 2015
Menze	120	41.36a–c	44.87a	55bc	58b
	240	35.62d–f	34.97d–g	38f–h	48c–e
	360	30.47f–h	31.50e–h	32hi	38f–h
Tsehay	120	36.62c–e	38.87b–d	67a	68a
	240	30.09gh	30.53f–h	47de	53b–d
	360	26.38hi	27.63h	37f–h	42e–g
ET-13	120	46.56a	42.27ab	52b–d	58b
	240	36.23c–e	27.02h	35g–i	44ef
	360	29.98gh	21.56i	29ij	22j
CV (%)		7.44		8.17	

Means followed by the same letter are not significantly different from each other at 5% level of significance according to Duncan's multiple range test

for all the varieties in both growing years. APE of all the varieties declined with increasing N rate. Increased N level reduced NUE in bread wheat varieties [44]. In general, the agro-physiological efficiency of the tested varieties showed a value less than 60 kg kg⁻¹, where this value was described as low which indicates a less managed system [43].

Apparent nitrogen recovery efficiency

Nitrogen apparent recovery (NAR) efficiency depends on the congruence between plant N demand and the quantity of N released from applied N [19]. The analysis of variance indicated that apparent recovery efficiency (RE) was significantly influenced by year, N rate, variety and the interaction of year × N rate, year × N rate × variety (Table 3). The highest apparent nitrogen recovery efficiency of 68% was recorded for variety Tsehay at N rate of 120 kg ha⁻¹ in the year 2015 which was statistically at par with that of 2014 under the same N rate for the same variety, while the lowest apparent recovery efficiency (22%) was recorded from variety ET-13 at N rate of 360 kg ha⁻¹ in 2015 which was statistically at par with that of 2014 under the same N rate for the same variety (Table 9). Similarly, Haile et al. [45] reported a decreasing trend in nitrogen use efficiency with increasing N rates with varieties tested. Likewise, Gauer et al. [43] reported the variation in nitrogen uptake efficiency of wheat which was ascribed to differences in climate, cultivar and nitrogen rates. The result further revealed that all bread wheat varieties showed a decline in ARE as the level increased

from 120 to 360 kg N ha⁻¹ in both growing years (Table 9). Moreover, Selamyihun et al. [46] reported increased in apparent nitrogen recovery efficiency at the rate of 110 kg N ha⁻¹ for wheat as compared to 60 and 85 kg N ha⁻¹. In general, the apparent recovery efficiency obtained from this study ranged between 68 and 22% for the different wheat varieties under the different N rates in both growing years. In contrast, [45] reported lower N use efficiency (27.10%) with the nitrogen rate of 120 kg N ha⁻¹ and the highest value (39.27%) at the lowest N rate of 30 kg N ha⁻¹ on bread wheat. In conformity with this result, studies from Ethiopia reported highest apparent nitrogen recovery efficiency of 46.8% [40], 65.8% [46] and 39.27% [45] on wheat in Ethiopia. However, the common apparent recovery N efficiency values ranging between 30 and 50%, and 50 and 80% indicate well-managed system [43].

Nitrogen harvest index

Nitrogen harvest index (NHI) is defined as the amount of N accumulated in grain divided by the amount of N accumulated in grain plus straw. Nitrogen harvest index indicates the level of efficiency of plants to use acquired nitrogen for grain formation [10]. A high NHI indicates efficient utilization of N. NHI was significantly influenced by N rate, variety as well as by the interaction of N rate × variety. However, the effect of year and the interaction of year × N rate and year × variety and year × N rate × variety were not significant (Table 3).

The maximum N harvest index (90.74%) was recorded from variety Menze at the control treatment which was statistically at par with variety Tsehay at the same treatment, whereas the lowest (74.1%) was recorded in by variety ET-13 at N rate of 240 kg ha⁻¹ which was statistically at par with the different N levels including the control for the same variety and with variety Menze at N rate of 120 and 360 kg N ha⁻¹ (Table 10). The two varieties (Menze and Tsehay) exhibited the highest NHI at the control treatment, whereas the rest N rates did not significantly affect NHI of the varieties which could be due to partitioning of the total nitrogen content more to the vegetative part of the crop than to the grain and increased the total aboveground biomass yield with the application of nitrogen. The present result is consistent with the study by Selamyihun et al. [46] who reported the decline in NHI at the highest N rate of 110 kg ha⁻¹. Sinebo et al. [34] reported nitrogen harvest index in durum wheat of 65%–82%, with an average value of 73% depending on N rate and timing of application.

Table 10 Interaction effect of N application rate and variety on nitrogen harvest index (NHI) of bread wheat at Enewari, central highland of Ethiopia

Variety	N rate (kg ha ⁻¹)	NHI (%)
Menze	0	90.74a
	120	80.1bc
	240	82.91b
	360	79.11bc
Tsehay	0	89.7a
	120	83.09b
	240	81.87b
	360	82.02b
ET-13	0	79.11bc
	120	78.89bc
	240	74.1c
	360	74.86c
CV (%)	4.21	

Means followed by the same letter are not significantly different from each other at 5% level of significance according to Duncan's multiple range test

Conclusion

From the study, it can be concluded that grain yields of Menze and Tsehay varieties were maximized with N application rates of 240 kg ha⁻¹ which were statistically at par, whereas the application of 360 kg N ha⁻¹ resulted in a decline in yield of variety ET-13 under suitable rainfall condition (2015). Among the varieties, Tsehay showed the highest grain and straw nitrogen concentration in the wetter and in the drier years. Moreover, varieties Tsehay and Menze showed higher nitrogen uptake as compared to variety ET-13 under the drier condition. Nitrogen fertilizer application led to a general decrease in nitrogen use efficiency traits in both growing seasons; these values were higher for the varieties tested in the second growing season. Moreover, Menze and Tsehay varieties (newly released) are superior in yield than variety ET-13 (old variety) and there was no significant difference among these varieties for most of the nitrogen use efficiency traits. Therefore, breeding objectives should not only focus on the improvement of grain yield but also on varieties which are efficient in utilization of the applied nitrogen.

Abbreviations

AE: agronomic efficiency; APE: agro-physiological efficiency; ARE: apparent recovery efficiency; GNC: grain nitrogen concentration; GNUM: grain nitrogen uptake; GY: grain yield; SNC: straw nitrogen concentration, NHI: nitrogen harvest index; NUE: nitrogen use efficiency; TNUP: total nitrogen uptake.

Authors' contributions

FB contribute to conception and design of the research proposal, data collection, analysis and interpretation of the data using SAS software version 9.3 and writing the manuscript. Prof. ND, Dr. AM and Prof. TT assisted in analysis and interpretation of the data and also in writing the manuscript article and revising it critically for final approval of the versions to be published. All authors read and approved the final manuscript.

Author details

¹ School of Plant Sciences, College of Agriculture and Environmental Sciences, Haramaya University, P.O. Box 138, Dire Dawa, Ethiopia. ² Chickpea and Malt Barley-Faba Bean Projects ICARDA, Addis Ababa, Ethiopia.

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Competing interests

The authors declare that they have no competing interests.

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