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Nitrogen Utilization Efficiency of Malt Barley (*Hordeum distichon* L.) Varieties as Influenced by Nitrogen Fertilizer Application Rates and Timing in Bassona Weranna District, Ethiopia's Central Highland

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Abstract

The experiment was conducted to assess the effects of nitrogen rate and time of application on nitrogen use efficiency (NUE) of malt barley varieties and their associations with grain yield and protein content. Factorial arrangement of randomized complete block design with three replications Four N rates (50, 70, 90, and 110 kg ha⁻¹), three times N application (T1 = full dose at sowing, T2 = 1/2 at sowing and 12 doses at mid-tillering, T3 = 1/4 dose at sowing, 12 doses at mid-tillering, and 1/4 dose at anthesis), and two malt barley varieties (IBONE 174/03 and EH 1847) were used in the treatment. The interaction effects of N rates with the time of application significantly increased grain yield from 2889.00 to 6611.10 kg ha⁻¹ with an acceptable range of grain protein contents of 9 to 12%. Varieties also significantly influenced the level of protein content. Though unacceptable in quality, the EH 1847 variety gave the highest grain protein content (12.21%), while the IBONE 174/03 variety gave an acceptable level of protein content (10.57%). N uptake increased along with N rates and times of application, which had a significant effect on grain yield. The straw yield was significantly affected by N rates, time of N fertilizer application, and variety. When compared to a single and two-time application, the three-time application of the highest N rate (110kg N ha⁻¹) improved grain N uptake, total N uptake, N use efficiency of grain, and N harvest index of malt barley.

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Introduction

Barley (*Hordeum vulgare* L.) is a cool-season crop that may be produced at altitudes ranging from 1500 to 3500 meters above sea level, however, it is most commonly produced between 2000 and 3000 meters (Lakew and Alemayehu, 1996). It grows better on alkaline soils than any other small grain crop and prefers well-drained soils (Birhanu *et al.*, 2005). Barely that isn't malting is widely

grown in regions where other cereals struggle to thrive, such as steep slopes, damaged soils, and areas with occasional drought and cold. Malting barley, on the other hand, requires a warm climate to produce a fat, mealy grain (Birhanu *et al.*, 2005).

Ethiopia is Africa's second-largest barley producer, after Morocco (total area coverage of 2.1 million hectares and total annual production of 2.16 million metric tons),

accounting for roughly 26% of the continent's total barley production; it is regarded as a centre of diversity, as its barley germplasm has global significance due to improved traits, such as disease resistance (Rashid *et al.*, 2019). With an area coverage of 959,273.36 ha and a total yearly production of around 2.03 million tons in the main cropping season, it is the fifth most important cereal crop in the country's domestic production, after tef, maize, sorghum, and wheat (CSA, 2018).

Malt barley is a high-opportunity crop in Ethiopia, with plenty of room for profitable expansion, especially when linked to the country's commercial brewing and value-added businesses (Berhanu, 2011). Malt is now the second greatest usage of beer in Ethiopia, and it is considered one of the country's cash crops, thanks to the rise of breweries and beer consumption levels (Kefale *et al.*, 2016); despite the country's favourable environment and prospective market opportunity, Ethiopian breweries import 69 percent of their malt from international sources (B. G. D. *et al.*, 2016).

The environment, genotype, and their interactions influence barley yield and end-use quality. Barley is extremely sensitive to nitrogen deficiency and highly responsive to nitrogen fertilizer (Alam *et al.*, 2007). Ethiopia Soils are nitrogen (N) and phosphorus (P) poor in most parts of Ethiopia. This is exacerbated by a lengthy history of farming with no NP replenishment, resulting in low soil fertility and crop yields (Jemal and Tadesse, 2020). The country has a high rate of nutrient depletion, with more than 60 kg of N, P, and K depleted per hectare each year, according to studies. (Debelle *et al.*, 2002). In Ethiopia, irregular seasonal rainfall, insufficient availability of other nutrients, nitrate leaching during the short but intense rainy seasons, ammonia volatilization, and continual removal in the highlands' grain mono-cropping systems could all contribute to inefficient N fertilizer use.

However, in Ethiopia in general, and the Amhara Region in particular, barely production is usually done with little or no external input, primarily in higher altitudes, steep slopes, degraded fields, or moisture stress zones (Alemu, 2001). Negassa *et al.*, (2007) looked into whether low barley yield was linked to low soil fertility in Ethiopia's highlands.

According to reports, nearly half of the N fertilizer applied to a crop is lost owing to N losses (Jamal *et al.*, 2006). According to Haile *et al.*, (2012), under ordinary field conditions, only 50 to 60% of applied nitrogen is

retrieved, and efficient timing and placement of N could boost applied nitrogen recovery by up to 70% or even 80%. The global nitrogen utilization efficiency (NUE) for grain production, including barely, is roughly 33 percent, according to Raun and Johnson, (1999).

The time and rate of nitrogen delivery are crucial for satisfying crop needs, and there are numerous options to improve N use efficiency (NUE) (Dhugga and Waines, 1989; Blankenau *et al.*, 2002). NUE is determined by the plant's growth stage at the time of application. Splitting N application into the later phases was found to be useful in increasing N uptake efficiency, according to reports (Krishnakumari *et al.*, 2000; Ashraf, 1998)

Variety can also differ in NUE as a result of differences in the absorption of nitrate. (Rodgers and Barneix, 1988) and N remobilization (Van Sanford and MacKown, 1986).

Improved cultivars, on the other hand, are produced without taking into account their ability to grow and yield in low-nutrient soils and are instead chosen for high yields under high-nutrient input circumstances (Van Sanford and MacKown, 1986).

According to Feil, (1992), varieties that produce a lot of biomass have greater Nitrogen use physiological efficiency (NUPE), which could lead to a fall in nitrogen use time efficiency (NUTE), lowering the total NUE of current varieties. Nitrogen use efficiency NUTE is heavily influenced by genetic variation (Singh and Arora, 2001), Harvest index (HI), and N biomass production efficiency are two factors that influence this (Ortiz-Monasterio *et al.*, 1997). However, because a loss in protein has been related to increased NUTE, selecting more HI may reduce the grain's N storage capacity. NBPE, on the other hand, has stayed relatively stable during genetic progress, according to reports (Ortiz-Monasterio *et al.*, 1997).

Malt barley is grown in Ethiopia during the rainy season, and losses of applied nitrogen through leaching can be reduced by applying nitrogen at the right rate and at the right time. Only a small amount of study has been done on the impact of N rate and application time on genetic variants in NUE. Such research could lead to a better understanding of how to increase grain yield by altering the rates and timings of nitrogen application. As a result, this research started to look at the differences in NUE traits and their relationship with grain production in malt barley types grown at various N rates and times.

Materials and Methods

Description of the experimental site

A field experiment was conducted in Bassonaworana district, during the main growing season. The geographical location of the experimental sites lies between 2019. 09° 03' 19.43" N latitude and 38° 30' 25.43" E longitude and altitude of 2807 m.a.s.l. The area has minimum and maximum average annual temperatures of 5.48°C and 20.99°C, respectively. The ten years' average annual rainfall ranges from 483.52 to 1071.30 mm, and the total annual rainfall during the main cropping season was 898.20 mm (data from Debre Berhan agricultural research centre metrological station (DBRC MS). The major crops grown in the area are Food barely, Potato, and Wheat The soil type of the area is Mesotrophic Vertisols (Esayas *et al.*, 2006).

Experimental Materials

The Malt barley varieties used in this experiment were IBONE 174/03 (V1) and EH 1847 (V2). The varieties are well-adapted and widely grown in the study area and nationally. Urea (46% N), triple superphosphate (TSP) with 46% P₂O₅, and Borax were used as the sources of nitrogen, phosphorus, and B & S, respectively.

Treatments and Experimental Design

A factorial combination of four N rates (50, 70, 90, and 110kg N ha⁻¹), two malt barley varieties (IBONE 174/03 and EH 1847), and three times of N application (T1 = Full dose at sowing, T2 = 1/2 dose at sowing and 1/2 dose mid-tillering, T3 = 1/4 dose at sowing, 1/2 dose at mid-tillering and 1/4 dose at anthesis) was evaluated in this study. Two additional treatments of zero kg N ha⁻¹ (controls), one for each variety were included in each replication for the evaluation of NUE and its traits in these two varieties. The treatments were laid in a factorial arrangement, using a randomized complete block design with four replications (4x3x2). The plot size was 3 m x 2 m. Spacing between plots and blocks was 0.5 m and 1 m, respectively. Each plot consisted of 10 rows 2 m in length with a spacing of 30 cm apart. The net harvested plot was 4.8m² (2.4mx2m) with eight rows with 2m length.

Experimental procedures

All field activities were carried out following the production package of the barely. Seeds of malt barley

varieties were sown over the well-prepared field in rows following the conventional time of sowing of the local farmers and availability of soil moisture at the recommended rate of 125kg ha⁻¹ in rows using hand drilling. Tri-Supper phosphate (TSP), Sulfur (S), and Boron in the form of Borax were applied in the rows at the rate of 16kg P ha⁻¹, 7 kg S ha⁻¹ and 0.1kg B ha⁻¹ in all treatment plots at the time of sowing (Molla, 2021). Times of N application were adjusted and applied according to Zadok's decimal growth stage for barley (Zadoks *et al.*, 1974) when the soil moisture is available for nutrient dissolution and absorption.

One chemical weeding was done 32 days after planting by using herbicide, Derby 175 SC for broad-leaved weed and Axial 045 EC for grass weed control at a rate of 100 ml and 1Lha⁻¹ per 200L of water respectively. To avoid damage and variability due to outbreaks of insect pests, which often occur in the area, insecticide (Karate 5% EC) was applied at the dose of 0.25Lha⁻¹ per 250L water before the start of shoot fly appearance which was a common insect pest.

Soil sampling and analysis

Soil samples were collected from (0-30 cm depth) randomly taken in a diagonal pattern using an auger and thoroughly mixed producing one composited sample. It was air-dried and passed through a 2 mm sieve for selected Physico-chemical properties mainly texture and soil total N, available phosphorus (Av. P), pH, organic matter (OM), organic carbon (OC), and cation exchange capacity (CEC) of the soil. The texture of the soil was determined by the hydrometer method (Bouyoucos, 1962). Total soil N was analyzed by the Kjeldahl digestion method with sulfuric acid (Jackson *et al.*, 1973). Soil pH was determined from the filtered suspension of a 1:2.5 soil-to-water ratio using a glass electrode attached to a digital pH meter, and a potentiometer (Motsara and Roy, 2008). Organic carbon content was determined by the volumetric method (Walkley and Black, 1934). Available P was analyzed by using Olsen's colorimetric method as described by Olsen (1954). The cations exchange capacity (CEC) of the soil was determined following the 1N ammonium acetate extraction (pH7) method.

Straw nitrogen and grain protein analysis

Plant samples collected at harvest were separated into grain and straw washed with distilled water, oven-dried at 60°C for 72h to a constant weight and the dry weight

measured using an electronic balance. Biomass and grain sub-samples were analyzed for total N content using micro-Kjeldahl digestion with Sulfuric acid as indicated in the FAO guide to laboratory establishment for plant nutrient analysis (Motsara and Roy, 2008).

The samples were ground by a rotor mill and allowed to pass through a 0.5 mm sieve to prepare a sample of 10 g. For the digestion with H₂ SO₄ (0.1 N) containing digestion mixture (Ten parts potassium sulfate and One-part copper sulfate), 1 g of each treatment's ground sample was used. Likewise, total grain N contents of treated and untreated plots were estimated from 1 g dry flour samples by the digestion method of Micro-Kjeldahl's apparatus according to the American Association of Cereal Chemists (AACC) (Bettge *et al.*, 2002).

Data measurements

Grain Yield (kg ha⁻¹)

It was measured from the harvested central unit areas of 2.1 m². The samples were cleaned following harvesting and threshing, weighed using an electronic balance, and adjusted to 12.5% moisture content.

Grain protein concentration (%)

The protein content of flour dry samples taken from the harvested grain yields of each treatment was calculated as Percentage protein = percentage N*6.25; where: 6.25 is the conversion factor (AACC, 2000).

Nitrogen use efficiency traits

Total N in the straw and grain samples was used to analyze N use efficiency and its component traits according to an expanded model of Moll *et al.*, (1982) and Ortiz-Monasterio *et al.*, (1997).

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$$\text{N uptake efficiency (\%)} = \frac{N_{tf}(xKgha^{-2}) - N_{tc}(Kgha^{-2})}{N_s(Kgha^{-2})} \times 100$$

Where N_{tf} = total aboveground N content at maturity of fertilized treatment

N_{tc} = total aboveground N content at maturity of control treatment

N_s = N supplied

$$\text{N Biomass production efficiency (kg kg}^{-1}\text{N)} = \frac{TDW_f(Kgha^{-1}) - TDW_c(Kgha^{-1})}{N_{tf}(Kgha^{-1}) - N_{tc}(Kgha^{-1})}$$

Where TDW_f = total dry weight of the fertilized treatment

TDW_c = total dry weight of control treatment

Nitrogen use efficiency (NUE, kg kg⁻¹) = $\frac{\text{Harvest index} \times \text{Production efficiency}}$

Nitrogen use efficiency_{grain yield} = N uptake take efficiency X N utilization efficiency

$$\frac{GDW_f(kgha^{-1}) - GDW_c(kgha^{-1})}{N_s(kgha^{-1})}$$

$$\text{N use efficiency}_{\text{grain protein}} = \frac{GNY_f(Kgha^{-1}) - GNY_c(Kgha^{-1})}{N_s(Kgha^{-1})}$$

$$\text{N harvest index} = \frac{GNY(Kgha^{-1})}{N_t(Kgha^{-1})}$$

Statistical analysis

Analyses of variances for the data measured were conducted using the SAS GLM procedure. The significant differences between treatments' mean values of each characteristic were computed using Duncan's Multiple Range Tests. Pearson's correlation analysis was done using Minitab software to determine associations between selected traits.

Results and Discussion

Soil Physico-chemical properties

Selected Physico-chemical properties were analyzed for composite surface soil (0-30 cm) samples collected from each replication before planting. The results indicated soil textural class of the study site was mainly clay-

dominated with a proportion of 50 % clay, 24% silt, and 26 % sand (Table 1), and its soil type is Mesotrophic Vertisols (Abayneh *et al.*, 2006). In line with this result, (Stewart *et al.*, 1970) stated that high clay content was due to high weathering, which arises from prolonged action or strong intensity of the weathering agents such as rainfall and temperature, finally, the oxide of Al and Fe was developed. To overcome the toxic effects of oxides of Al and Fe, the incorporation of organic matter resources into these soils would improve soil fertility, aeration, and nutrient availability for sustainable crop production (Stewart *et al.*, 1970).

According to the soil pH rating of Tadesse *et al.*, (1991), the soil reaction (pH) of the experimental field falls in the range of moderately acidic (5.88). However, according to the Regassa and Agegnehu (2011) barley crop package, soil pH values in the range of 6.5 to 7.8 are the most suitable for barley production. So, the pH result of this research site requires due attention to improve its acidity problem to ideal ranges for optimum plant growth and availability of most plant nutrients through liming. Compared with this fact the availability of most plant nutrients like N, K, and S, appears to be more highly affected directly by soil pH than many others. low (2.21%) level of soil organic matter was recorded, which might have contributed to a relatively low (0.2%) level of total N in the soil due to the decomposition and utilization of organic carbon by the growing crop. Brady and Weil (2002) concluded that tillage and weeding aerate the soil and break up the organic residues, making them more susceptible to microbial decomposition. Tisdale *et al.*, (2002) indicated that OC influences many soil biological, chemical, and physical properties that favourably influence nutrient availability. However, Esmailzadeh and Ahangar (2014), showed that soil with over 2% of organic matter content was good for plant growth and development. The total N (0.2%) ranged nearly low based on the classification of Tadesse *et al.*, (1991). This was due to the leaching of soil N due to high rainfall intensity during the cropping season, continuous uptake of N by previous crops, poor soil fertility management of the field (lack of manuring, crop rotation, fallowing) may result in low total N level in the soil. Regarding N economy, the soil of the study area requires further improvement of the fertility status of the soil by applying organic or inorganic N sources, crop rotation, and other soil management techniques. Because the farming field might be subjected to intensive farming and high rainfall that may cause soil organic matter oxidation and leaching of exchangeable basic cations resulting in the

reduction of soil organic matter contents and total N (Wakene and Heluf, 2003). The available phosphorus of the experiment is in the range of low (7.84mg kg^{-1}) based on the rating of Olsen (1954). This might be the result of different soil misuse and management practices specifically the type, and rate of organic and inorganic fertilizers utilized in cultivated land. This has coincided with the research findings of Wakene and Heluf (2003); Donis and Assefa (2017) who stated that insufficient application of organic nutrient sources, removal of crop residues from cultivated land, intensive and continuous cultivation of the land causes low total N, low amount of phosphorus content in the soils that affect the soil productivity due to the removal of exchangeable basic cation like Ca^{+2} through leaching in high rainfall areas; causes phosphorus fixation, forced oxidation of OC and thus resulted in a reduction of total N. This is also confirmed by the findings of Láng *et al.*, (2013); Tilahun (2007), a low amount of phosphorus content due to repeated cultivation of the field and removal of basic cations causes phosphorus fixation. The CEC of the soil sample was medium ($20.72\text{cmolc kg}^{-1}$), as per the rating established by Landon (1991).

The effect of nitrogen application rate and timing on grain yield

Grain yield was significantly ($P < 0.001$) affected by N rates and time of N application and the interaction of N rate with the time of application. However, the main effect of variety and its interaction effects with time and rate of N fertilizer application and the combined (three-way) interaction effects had non-significant responses to the grain yield of malt barley variety (Table 2).

The time of N fertilizer application at T3 exhibited a 44% additional (1461kg ha^{-1}) grain yield increment as compared to the lower mean grain yield (3327.30kg ha^{-1}) which was obtained from N fertilizer application at the time of T1. On the other hand, higher (110kg ha^{-1}) rates of N fertilizer application recorded 60.4% (1929.4kg ha^{-1}) additional grain yield increment as compared to the lowest (50kg ha^{-1}) rate of N fertilizer, which produced the lower (3194.4kg ha^{-1}) grain yield (Table 2). Studies by Gauer *et al.*, (1992) and Ali (2010) agreed with the result, as it was reported that there were positive and linear responses of grain yield to incremental rates of N fertilizer. The higher (6611.10kg ha^{-1}) grain yield of malt barley resulted from the interaction of a higher (110kg ha^{-1}) N rate at the time of application T3, while the lower (2889.00kg ha^{-1}) grain yield resulted from the interaction of a lower (50kg ha^{-1}) N rate at the time of application T1

(Figure 3). The result showed that the interaction of the higher N fertilizer rate with the time of application T3 recorded 128.8% (3722.1kg ha⁻¹) of additional yield increment as compared to the lowest rate of N fertilizer at the time of application T1. The higher response of grain yield to the highest N rates in this study might be attributed to the splitting of higher rates of N fertilizer into three parts, which enables efficient utilization of malt barley varieties and stimulates the production of the higher grain yield by influencing other yield components favourably. This result is due to the grain yield's response to the higher N rates (110kg ha⁻¹) and time of application, T3.

This result agreed with Hussein *et al.*, (2015), who reported that splitting the application of N at a higher rate resulted in a consistent grain yield increment as the time of N application went from T2 to T3 and T4, respectively than when N was applied once. This may be attributed to the synchrony between the time of high need for the plant for N uptake and the time of availability of enough N in the soil at the specified growth stages. According to Hussein *et al.*, (2015), the yield-enhancing effect of the four split applications of N is attributed to a reduction in the loss of nitrate by leaching during the wet growing season.

Thus, at a time of high need for N, the plant may have taken most of the nitrate from the soil, leaving less of it available for leaching by the percolating rainwater, especially in high rainfall areas where nitrate leaching is very common. With the splitting of higher rates of N fertilizer, N availability matched crop needs during the growing period (Hussein *et al.*, 2015), splitting N application rates into three or four applications increased grain yield by 3.1 and 3.6 times that of control and 1.5 and 1.6 times that of one application, respectively. The same trend was also found for straw and biological barley yield (Hussein *et al.*, 2015). However, this finding

is in contrast with results obtained by Abebe and Manchore (2016), who stated that to maximize the grain yield of cereal crops, applying 69kg ha⁻¹ N is appropriate as compared to 92kg ha⁻¹ N by using the split application because the well-balanced supply of N at an optimal amount results in a higher net assimilation rate and increased grain yield of cereal crops.

Grain protein content

Grain protein content (GPC) of malt barley was significantly influenced by the main effects of N rate, varieties, the interaction effect of the time of N fertilizer application, and variety, but it had non-significant responses as a result of the main effect of time of N fertilizer application and the rest (TxNr, NrXV, and NrXTxV) of the interaction effects (Table 3). The higher (11.81%) grain protein content was recorded from the higher N rate of N fertilizer application (110kg N ha⁻¹) whereas the lower (11.20%) grain protein content was recorded from the lower (50kg ha⁻¹) N rate. Likewise, the higher (12.21%) grain protein content was recorded from the EH 1847 variety while the lower grain protein content (10.57%) was recorded by the IBONE 174/03 variety of acceptable quality standards. These GPC differences among varieties might be due to the genotype differences of the varieties. This result agrees with those who reported that the grain protein content of malt barley was significantly influenced by the main effects of N fertilizer levels and varieties, while their interaction effect responded non-significantly. Similar to Beauchemin *et al.*, (1995), they found that the protein content would increase with an increased rate of N. However, the protein content would increase at a slower rate. However, too much N can increase protein beyond levels set by the maltsters. Increased protein content may lengthen steeping times (slows water uptake during steeping), make germination more erratic, and result in undesirable malt qualities (Johnston *et al.*, 2007).

Table.1 Physico- Chemical properties of the soil.

Physical properties				Chemical properties						
Clay	Silt (%)	Sand (%)	Textural class	CEC Cmol(+)kg ⁻¹	PH (1:2:5 (H ₂ O)	TN (%)	OC (%)	C/N	Av.P mg/Kg	OM (%)
50	24	26	Clay	20.72	5.88	0.2	2.21	7.89	8.04	3.8

CEC = Cation Exchange Capacity, C/N= Carbon to Nitrogen ratio, OC = Organic Carbon, OM = Organic Matter, TN = Total Nitrogen, Av.P = Available Phosphorus, cmolkg⁻¹ = Centimole cation per kilogram of soil

Table.2 Effect of rates and time of nitrogen application on yield and yield components of malt barley varieties

N rate (kg ha ⁻¹) (NR)	GY
50	3194.40 ^d
70	3832.20 ^c
90	4213.00 ^b
110	5123.80 ^a
N application time (NT)	
T1	3327.30 ^c
T2	4157.10 ^b
T3	4788.20 ^a
Variety (V)	
IBONE 174/03	4218.70 ^a
EH1-1847	3963.00 ^a
Time	***
varieties	NS
N Rate	***
T X V	NS
T XNr	***
V xNr	NS
T Xv x Nr	NS
CV	13.60

Means followed by the same letter within a column are not significantly different at a 5% level of significance by DMRT, *, ***, and NS showed significant differences at 0.05 and 0.001 Probability levels and non-significant differences, respectively. Where, T1= full at sowing, T2= 1/2 at sowing and 1/2 at mid tillering, T3=1/4dose at sowing, 1/2dose at mid tillering, and 1/4at anthesis; CV = Coefficient of variation.

Table.3 Effect of rates and time of nitrogen application on the grain protein content of malt barley varieties

N rate (kg ha ⁻¹) (NR)	GPC
50	11.20 ^b
70	11.33 ^{ab}
90	11.22 ^b
110	11.81 ^a
N application time (NT)	
T1	11.23 ^a
T2	11.42 ^a
T3	11.52 ^a
Variety (V)	
IBONE 174/03	10.57 ^b
EH1-1847	12.21 ^a
Time	NS
varieties	***
N Rate	*
T X V	***
T XNr	NS
V xNr	NS
T Xv x Nr	NS
CV	6.42

Means followed by the same letter within a column are not significantly different at a 5% level of significance by DMRT, *, **, ***, and NS showed significant differences at 0.05, 0.01, 0.001 Probability levels and non-significant differences, respectively. Where, GPC=Grain Protein content T1= full dose at sowing, T2= ½ dose at sowing and ½ dose at mid tillering, T3=1/4 dose at sowing, ½ dose at mid tillering and ¼ dose at anthesis; CV = Coefficient of variation.

Table.4 Effect of rates and time of nitrogen application on nitrogen uptake traits of malt barley varieties

N rate (kg ha ⁻¹) (NR)	GNUp	SNUp	NtUp	NHI
50	57.36 ^c	27.43 ^b	84.78 ^c	0.68 ^b
70	69.33 ^b	26.00 ^b	95.30 ^c	0.72 ^a
90	76.00 ^b	36.00 ^a	112.00 ^b	0.67 ^b
110	96.71 ^a	35.00 ^a	131.61 ^a	0.73 ^a
N application time (NT)				
T1	59.70 ^c	26.57 ^b	86.26 ^c	0.70 ^a
T2	76.10 ^b	32.00 ^{ab}	108.04 ^b	0.70 ^a
T3	88.76 ^a	34.65 ^a	123.40 ^a	0.71 ^a
Variety (V)				
IBONE 174/03	72.35 ^a	30.37 ^a	102.72 ^a	0.70 ^a
EH1-1847	77.34 ^a	31.74 ^a	109.08 ^a	0.70 ^a
Time	***	*	***	NS
varieties	NS	NS	NS	NS
N Rate	***	***	***	**
T X V	NS	NS	*	NS
T XNr	***	NS	***	*
V xNr	NS	NS	NS	NS
T Xv x Nr	NS	NS	NS	NS
CV	16.56	30.87	15.72	8.78

Means followed by the same letter within a column are not significantly different at a 5% level of Significance by DMRT, *, *** and NS showed significant differences at 0.05 and 0.001 Probability levels and non-significant differences, respectively. Where, GNUp=Graine nitrogen uptake, SNUp= Straw nitrogen uptake, NtUp= Total Nitrogen uptake, T1= full dose at sowing, T2= ½ dose at sowing and ½ dose at mid tillering, T3 = ¼ dose at sowing, ½ dose at mid tillering and ¼ dose at anthesis; CV = Coefficient of variation.

Fig.1 Interaction effect of nitrogen rates and time application on grain yield

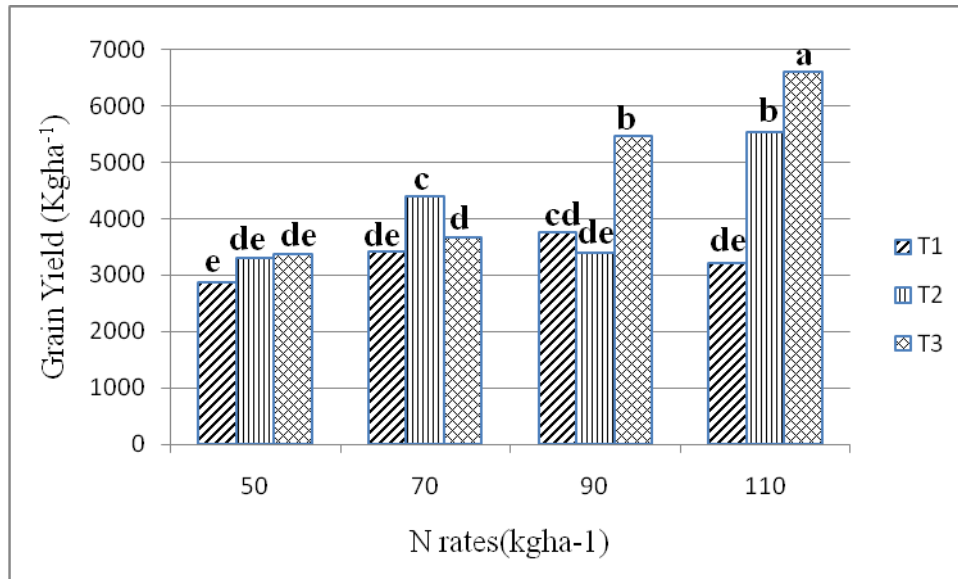


Fig.2 Interaction effect of variety and time of nitrogen application on grain protein content.

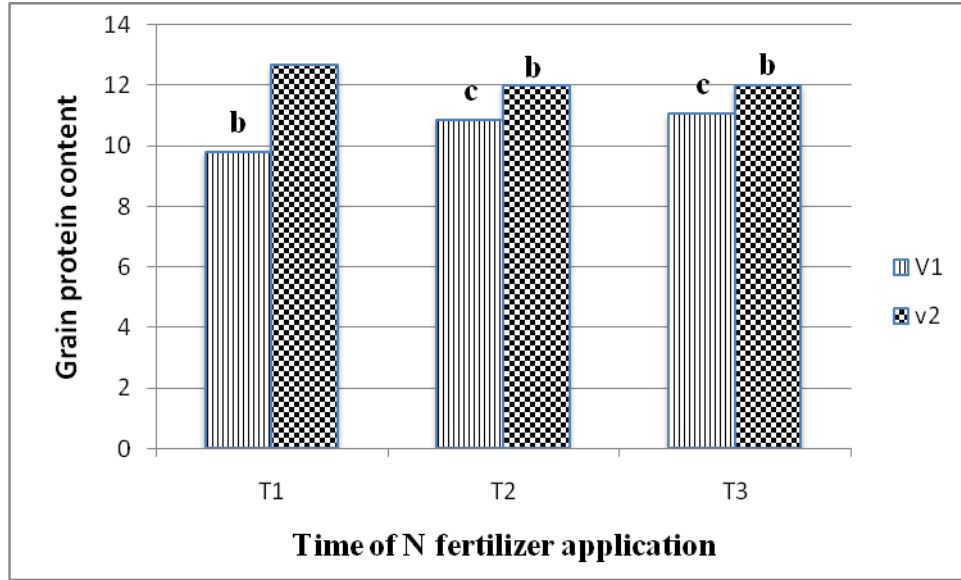


Fig.3 The interaction effect of nitrogen rate and time of application on grain N uptake

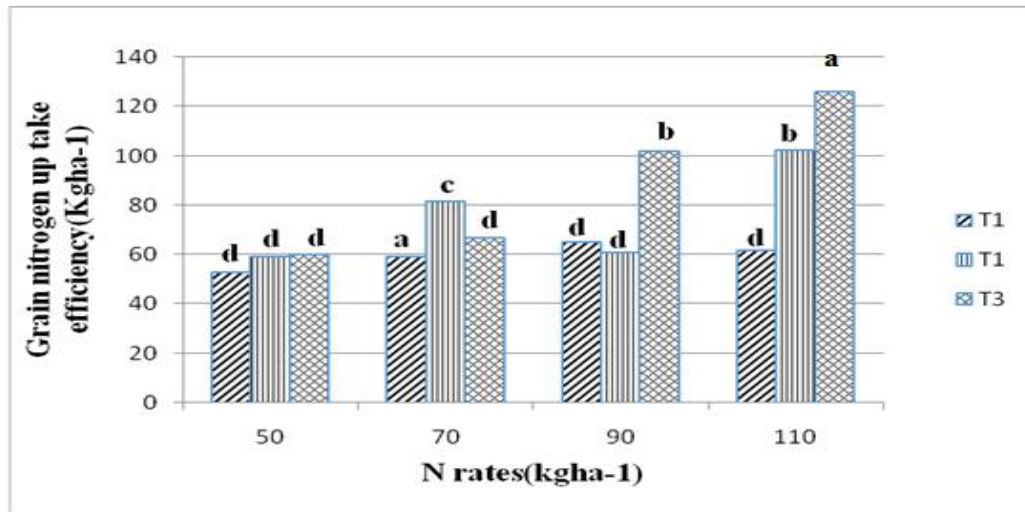


Fig. 4 The interaction effect of nitrogen rates and time of application on total nitrogen uptake

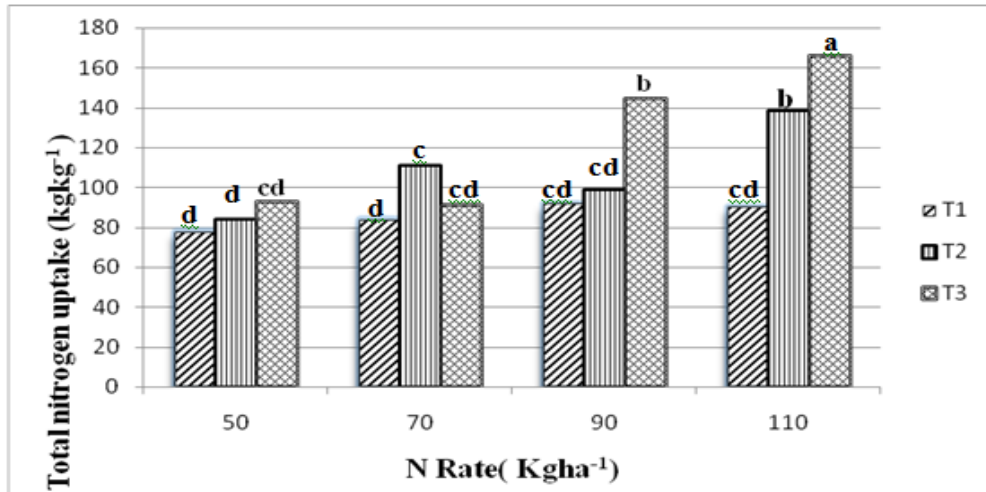


Fig.5 The interaction effect of time of nitrogen fertilizer application and variety on total nitrogen uptake

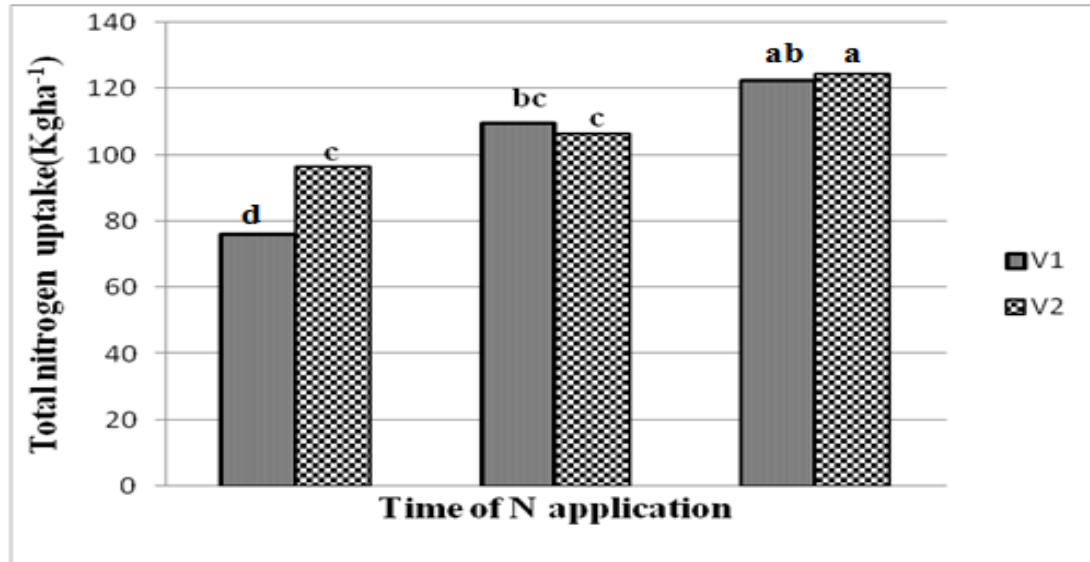
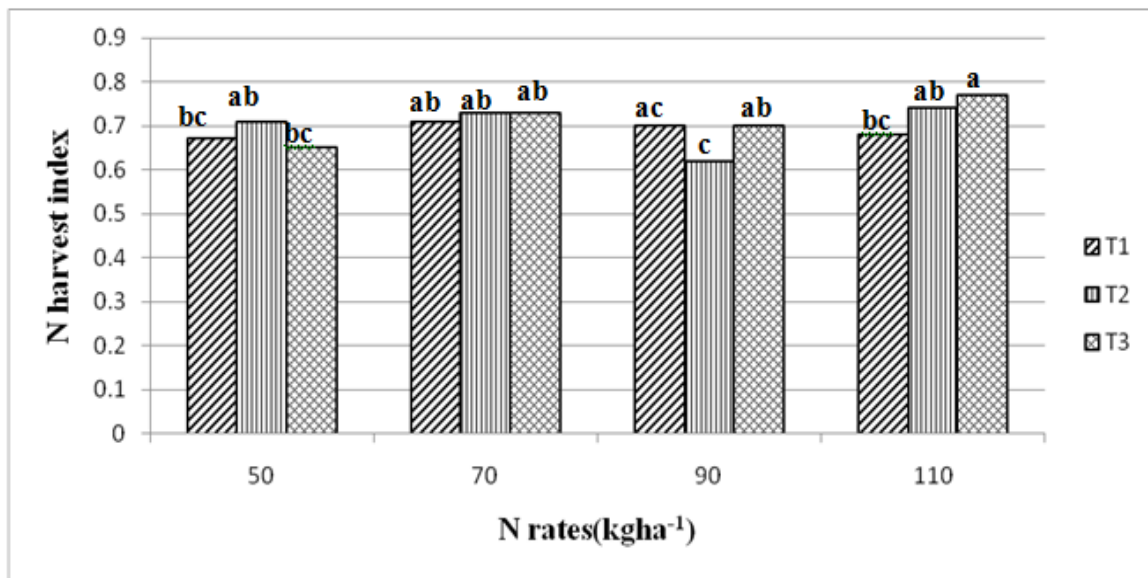


Fig.6 The interaction effect of nitrogen rates and time of nitrogen fertilizer application on the nitrogen harvest index



In line with the result of this study, both the main effect of N fertilizer rate and varieties had grain protein content within the acceptable range of malt quality, except for the EH 1847 variety (Appendix table 1), where it was declared by the Ethiopian standard authority (Liben *et al.*, 2011), Assella malt factory (AMF, 2012) (Appendix Table 1) and Gondar Malt Factory (GMF, 2015) (Appendix Table 2) that the protein level of the raw barley quality standard for malt should be between 9 to 12%.

The interaction effects of time of N fertilizer application with variety also revealed that variety EH 1847 exhibited

the highest (12.67%) range of protein content when it was applied at T1. On the other hand, the EH 1847 variety had a lower (12.00%) percentage of protein content when N fertilizer was applied to T2 and T3 (Figure 4). IBONE 174/03, on the other hand, showed a lower (9.80%) and higher (11.07%) percentage of protein content when interacting with the time of N fertilizer application (T1 and T3, respectively) (Figure 4). Based on the time of N fertilizer application, the two studied varieties showed different trends in their protein concentration.

According to Hussein *et al.*, (2015) and Brian *et al.*, (2007), grain protein content was found to be significantly influenced by time of application; where the higher grain protein concentration was obtained under four splits of N, while the lower grain protein concentration was obtained due to early N application at planting and tillering compared to split N application up to anthesis; this truth is agreed with the case of IBONE 174/03 but not true for the EH 1847 variety. Hence, in line with this, the variation in grain protein content of the two varieties might be due to the genetic variation between them. This result agrees with Beauchemin *et al.*, (1995), who reported that there was a variation in grain protein content due to genetic variation of malt barley varieties.

On the other hand, according to the Ethiopian standard authority (Liben *et al.*, 2011), Assella malt factory (AMF, 2012)(Appendix Table 1), and Gondar malt factory (GMF, 2015)(Appendix Table 3), the protein level of the raw barley quality standard for malt should be between 9 and 13%. In line with the results of this study, the main effects of variety IBONE 174/03 and its interaction effects with the time of N fertilizer application had grain protein content within the acceptable range, whereas the EH 1847 variety exhibited unacceptable ranges of protein content (Appendix table 1). An increase in protein may increase steep times, and create undesirable qualities in the malt, excessive enzymatic activity, and low extract yield (Johnston *et al.*, 2007). It also slows down water uptake during steeping, potentially affecting the final malt quality.

Effects of nitrogen rate and time of application and Verities ongrain nitrogen uptake efficiency

It was affected significantly ($P < 0.001$) by the main factors of N rates and time of application, but the variety had a non-significant effect on grain N uptake (GNUp) of the malt barley variety. The interaction effects of N rate with the time of application also significantly ($p < 0.001$) affected the grain nitrogen content of the crop, while the interaction effects of Nr x V, TxV, and Nr x T x V had a non-significant response to GNUp. The highest (96.71%) and the lowest (57.36%) grain N uptake were recorded at the higher (110kg ha⁻¹) and lower (50kg ha⁻¹) rates of N fertilizer. The main effect of time of N fertilizer application also revealed the higher (88.76%) and lower (59.70%) grain N uptake when it was applied at the time of T3 and T1, respectively (Table 4).

The result of the interaction effect of N rates and time of application indicated that the higher (126.22%) and the lower (53.00%) grain N uptake was recorded when the higher (110kg ha⁻¹) and the lower (50kg ha⁻¹) N rates interacted with T3 and T1, respectively (Figure 5). This might be due to the split application of higher levels of N rate three times, which enables the crop to uptake the available nitrogen easily from the soil to yield a higher amount of grain.

The result agrees with Hussein *et al.*, (2015), who reported that GNUp was significantly affected by N fertilizer rate and timing of application. It was noticed that the amount of N uptake by barley yield with 100kg ha⁻¹ and four times of application was higher than that of 70kg ha⁻¹ which was added once at pre-planting. Thus, splitting N treatments yielded more N uptake by the grain than applying pre-plant treatment. These findings confirm the importance of three-split applying N to yield more GNUp.

Effects of nitrogen rate and time of application and Verities on nitrogen uptake efficiency of Straw

Straw N uptake (SNUp) was affected significantly by the main effects of N rates ($p < 0.001$) and time of application ($P < 0.05$). However, the main factor variety and two and three-way interaction effects had no significant effect on straw N uptake of malt barley varieties.

The higher and lower N uptake were recorded at the higher and lower rates of N fertilizer. The timing of N fertilizer application revealed higher (34.65%) and lower (26.57%) N uptake of the straw when N fertilizer rates were applied at T3 and T1, respectively, but with statistically similar results at N rates of 110 kg ha⁻¹ and 90 kg ha⁻¹ (Table 4). This might be due to the split application of the higher N rate, which enables the crop to uptake the available nitrogen easily from the soil to yield a higher amount of straw. This result disagrees with the result of Hussein *et al.*, (2015), as it was reported that SNUp was significantly affected by N fertilizer rate and timing of application with values of N uptake by barley straw shares of 18 to 31% of its uptake by grains. The maximum straw N uptake was obtained at the highest N rate (100kg N ha⁻¹) with the application of N at two splits (two weeks + four weeks from sowing). However, the other split applications did not show a significant increment as the rates of N increased.

Effects of nitrogen rate and time of application and Varieties on total nitrogen uptake efficiency

Total N uptake (NtUp) was affected significantly ($P < 0.01$) by the main effect of N rates and time of application, but variety had a non-significant effect. The interaction effects of time of N fertilizer application with variety and N rate also significantly ($P < 0.05$ and $P < 0.001$) affected the total nitrogen content of the crop, while the interaction of N rate versus variety and the three-way interaction had no significant effect on NtUp of the crop.

The higher (131.61%) and the lower (84.78%) total N uptake was recorded at the higher (110kg ha⁻¹) and lower (50kg ha⁻¹) N rates. The main effect of time of N fertilizer application also revealed the higher (123.4%) and the lower (86.26%) NtUp when it was applied at the time of T3 and T1, respectively (Table 4).

The results of the interaction effect of N rates and time of application indicated that the higher (165.87 %) and the lower (78.00%) total nitrogen uptake were recorded when the higher (110kg ha⁻¹) and the lower (50kg ha⁻¹) N rates were applied at T3 and T1, respectively (Figure 6).

The interaction effects of time of N fertilizer application and variety indicated that the higher (122.43 %) and the lower (76.10%) total N uptake was recorded in the IBONE 174/03 variety when N fertilizer was supplied at the time of application, T3 and T1, respectively (Figure 7). Similarly, varieties EH 1847 and EH 1848 had the highest (124.37%) and lowest (96.10%) total N uptake during N fertilizer application, T3 and T1, respectively.

Generally, EH 1847 exhibited the highest NtUp as compared to the IBONE 174/03 variety throughout the extended supply of N fertilizer at the time of T1 and T2, respectively

Nitrogen harvest index

In cereals, the N harvest index, which is defined as the ratio of N in grain to total N uptake, is a crucial factor to consider. The NHI represents the grain protein concentration and, as a result, the nutritional quality of the grain (Hirel *et al.*, 2007).

The nitrogen harvest index (NHI) was significantly ($P < 0.001$) affected by the main effects of N rate and the interaction effect of N rate with the time of N application ($P < 0.01$) but not significantly affected by the main

factors of time of N application, variety, and the interaction effects of time of application and variety, variety and N rate, and the three-way interaction responses. Even if 50 and 90, 70, and 110kg N ha⁻¹ revealed statistically similar results, the higher NHI (0.73) was recorded at 110kg N ha⁻¹ and the lower NHI (0.67) was obtained at 90kg N ha⁻¹ (Table 4). Results of the interaction of N rate with the time of application indicate that the higher (0.77) NHI was recorded from the interaction of 110kg N ha⁻¹ at the time of application T3 and the lower (0.62) NHI resulted from the 90kg N ha⁻¹ at the time of N fertilizer application T2 (Figure 8).

The higher (0.77) NHI in the three split applications of the highest N rate (110kg ha⁻¹) in this study is consistent with the findings of Jan *et al.*, (2010) and Haile *et al.*, (2012), who reported that there was a higher NHI in the three split applications compared with the two split applications (higher index with the additional N application at anthesis), where N applications of 1/4 doses at sowing, 1/2 doses at mid-tillering, and 1/4 at anthesis resulted in 5.8% higher total plant N partitioning in the grain than the applications of 1/2 doses at sowing, and 1/2 doses at mid-tillering. Trends of this research work also indicated that there was an increment in NHI as the N rate increased from 50kg N ha⁻¹ to 110kg N ha⁻¹ and as the time of N fertilizer application went from T1 to T3 (Figure 8). Therefore, the result agreed with Fageria and Baligar (2005) and Erkeno (2014), who reported that the N harvest index (NHI) increased with increases in N rates and time of application. However, the result is consistent with the study by Kidanu *et al.*, (2000) and Belete *et al.*, (2018) who reported that there was a decline in NHI at the highest N rate of 110 kg ha⁻¹ in cereal crops as compared to 70 kg ha⁻¹ regardless of the time of application.

Some of the key restrictions limiting malt barley production in Ethiopia include low accessible soil nitrogen and lower plant NUE. Providing the crop with a well-balanced supply of nitrogen could result in improved grain production.

The results of this experiment revealed that malt barley grain yield was increased with increasing N fertilizer rates in the three-split application while grain yield became low at a point of low N rate but with acceptable protein content at all times of application. 110kg ha⁻¹ N rates and the IBONE 174/03 variety with a three-split application (1/4 doses at sowing, / doses at mid-tillering, and 1/4 doses at anthesis) could produce an optimum grain yield with the required quality. Low N rates (50 kg

ha⁻¹) result in lower grain yields but with acceptable quality.

The improved variety produced higher grain proteins at the highest N rate. Accordingly, this result indicated that increasing N rates increases the concentration of N but still with acceptable grain protein content. IBONE 174/03 and EH 1847 varieties exhibited 10.57 and 12.21% grain protein content, which was within the acceptable recommendation ranges of the EQSA, AMF (Appendix Table 1), and GMF protein levels (9 to 12%)(Appendix Table 3) but an unacceptable grain protein content in the case of the EH 1847 variety.

The timing of the N fertilizer application had a non-significant effect on the grain protein content of the two varieties. From the interaction effects of variety with N rate, it had a significant effect, but the N rate with the time of application for two varieties was non-significant on grain protein content. The interaction effects of time of N fertilizer application with variety affected significantly the grain protein contents of malt barley varieties.

From this study, it is evident that split N applications (1/4 at the time of sowing, 1/2 at mid tillering and 1/4 at anthesis) of the highest N rate (110kg N ha⁻¹) generally improved grain N uptake, total N uptake, N use efficiency of grain and the N harvest index of malt barley. Treatments with three split applications of N significantly increased most of the parameters of N uptake and N use efficiency of malt barley compared to a single application at sowing. Generally, the results indicated that N fertilizer rates and times of application markedly affected the yield performance and N use efficiency of the improved variety.

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