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Genetic Variation in N-Use Efficiency of Malting Barley Genotypes

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Abstract An experiment was carried out in 2010 at Adet, in Northwest Ethiopia, to differentiate the genetic potential of malting barley genotypes in Nitrogen Use Efficiency (NUE), to assess the effect of nitrogen labels and genotype by nitrogen interaction. Nine malting barley genotypes at two levels of nitrogen were evaluated as a randomized complete block design with four replications. Results showed highly significant variation among the genotypes for NUE and yield traits studied. The genotypes EH1603-F5.B1-4, HB-1533 and EH1609-F5.B3-10 had the highest NUE and were higher yielding. The presence of such variation indicates that efficient and high yielding malting barley genotypes can be directly selected. Highly significant variation was observed between N-levels for most of the NUE traits in which higher value was obtained from the lower level of N. Therefore to be economical as well as to prevent environmental effect of N, it is better to use the lower rate of N. There was no significant interaction between N-levels and genotypes. Generally choosing efficient genotypes and the application of low levels of N seems to be the most efficient way to supply nitrogen when grain yield, NUE, and quality are our goals. High phenotypic coefficient of variation (PCV) was observed for UPE (34.57%) and NUE (31.6%). High genotypic coefficient of variation (GCV) was not observed for the studied traits. Of the traits of malting barley studied in this experiment, 50% show low heritability which makes selecting for these traits difficult because environmental effect is more evident that genetic effect. Therefore future breeding efforts in malting barley should aim at exploiting the genetic variability available in the test genotypes.

Keywords Malting barley, Nitrogen Use Efficiency, Genetic variability, Grain yield

1. Introduction

Barley (*Hordeum vulgare* L.) is the fourth grain crop both in area and production in the world after maize, wheat and rice[1]. In Ethiopia, barley is an important cereal crop that is mainly grown by subsistence farmers[2]. It is grown in wide range of environments with altitude of 1500 and 3500 m above sea level, but predominantly grown from 2000 to 3500 m above sea level[3]. Diverse landraces and morphological classes of barley are adapted to specific sets of agro-ecological and microclimatic regimes throughout the country.

Barley is used as food, feed, malt and industrial purpose. Malted barley is the source of sugars, which are fermented into beer. Nitrogen is the key nutrient input for achieving higher yield of barley. Grain protein is the key factor that determines malting barley quality. Barley requires far less water and can be cultivated in areas where irrigation water is less easily obtainable. Nitrogen is the key nutrient input for achieving higher yield of barley. It is very sensitive to insufficient N and very responsive to N fertilization. Farmer use nitrogen fertilizers indiscriminately without adequate information concerning actual soil requirements. This results in over application of some nutrients or under application of others that will result in deficiency of their use. Enormous efforts are therefore needed to formulate nitrogen recommendations especially for malt barley.

The main farmers' instrumentation to meet quality parameters of cultivated malting barley is to decrease the amount of applied N fertilizer. However, this strategy does not allow producing high yields of grain. On the other hand, increasing levels of applied nitrogen and/or high soil nitrogen levels increase protein contents in the grain of cereals. Arends et al.[4] have reported an effective approach to balance the conflict between high yield and malt quality on nitrogen application is to develop cultivars with grain protein concentration that are less sensitive in terms of grain protein accumulation to variable levels in soils.

The efficiency with which nitrogen (N) is used by malting barley and other cereals is gaining importance, because of increasing costs of N fertilizer, environmental contamination and deterioration of malt quality as N is increased. High yielding nitrogen efficient genotypes of any crops may

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increase production, reduce costs and risk, and minimize the potential negative effects of N use on the environment. Grain yield being the final economic product, N Use Efficiency (NUE) has usually referred to the relationship between grain yield and fertilizer or total available N. Nitrogen uptake efficiency refers to the ratio of N in the biomass to the N in fertilizer and/or soil while N utilization efficiency is the amount of grain yield produced per unit of N recovered in the biomass[5].

Although soil fertility research programs have been successful in establishing fertilizer N optimums for selected cereals, little work has been done to improve genetic NUE in malting barley. Therefore, plant breeders need to develop cultivars that can absorb N more efficiently from the soil and effectively partition absorbed N to the grain. Such cultivars could minimize loss of nitrogen from the soil and make more economic use of the absorbed N[6]. 17 barley genotypes were also compared in their NUE and reported that barley genotypes differed in their N-use traits indicating that there exists some further potential to improve NUE through crop management and plant breeding[7]. Twenty six Ethiopian barley genotypes were compared and reported that there exists genetic variation for NUE[8]. More studies are required to identify malting barley genotypes, which maintain high yield potential with low N fertilizer requirements keeping their quality in accepted level. With this regard, Information on grain yield and NUE of malting barley genotypes under northwestern Ethiopian growing conditions is scarce.

Therefore, the present study was conducted to differentiate the genetic potential of malting barley genotypes in nitrogen use efficiency (NUE), to assess the effect of nitrogen labels and to assess the effect of genotype by nitrogen interaction.

2. Materials and Methods

The experiment was carried out during the 2010 growing season in the experimental field of Adet Agricultural Research Center (AARC). The center is located at 37°29 ′E and 15°16 ′N in the Amhara National Regional State, Ethiopia. The center is found at an altitude level of 2240 m above sea level and receives rainfall of 1230 mm per annum. The soil type is vertisoil and drained Nitosol with a pH of 6.0.[9]. The average annual maximum temperature of AARC is 25.5°C and the average minimum temperature is 9.2°C. The main rainy season extends from June to October.

The experiment was carried out with nine malting-barley genotypes where two (EH1603-F5.B1 and EH1609-F5.B3-10) are promising and seven (Arna, Beka, HB-52, HB-120, HB-1533, Holker and Miscale-21) are released varieties under two N-levels. Nitrogen levels of 20.5 and 41 kg ha^{-1} from origin of urea was used as N treatments. A complete factorial arrangement of treatments (N-level × genotype) as in a randomized complete block design (RCBD) with four replications was used as a design.

To avoid N losses by leaching, urea applications were split in three identical quantities and applied at the stages of sowing, tillering and at the start of flowering while all the DAP was applied once during sowing for all treatments. The experiments were hand-sown at a seeding rate of 125kg/ha on 23 June 2010 in plots of six rows, 0.2 apart and 2.5m long. The distance between plots and replications were 0.4 and 1.5m, respectively. The central four rows were used for all data collection including final yield. Weeds were annually removed throughout the growing season. Agronomic and malting quality traits data were collected from field and laboratories.

Nitrogen use efficiency was analyzed according to an expanded model of Moll et al.[5], while that of N uptake efficiency was obtained as ratio of total plant N to N supply per plot. The N contents of different plant parts were estimated following a modified version of the kjeldahl procedure using a nitrogen auto analyzer[10]. Analysis of variance (ANOVA) was conducted using Statistical Analysis Software (SAS) computer software program following SAS statement for randomized complete block design in factorial experiment[11].

3. Results and Discussion

3.1. Agronomic Traits

There was significant genotypic variation among genotypes for all the agronomic traits studied. For instance, the yield ranged from 2530 kg ha^{-1} to 3640 kg ha^{-1} of which EH1609-F5.B3-10 (3640 kg ha^{-1}), HB-1533 (3400 kg ha^{-1}) and EH1603-F5.B1-4 (3030 kg ha^{-1}) were top yielding genotypes. The genotypes varied significantly ($P < 0.0001$) for plant height ranging from 81 to 107 cm with a grand mean of 94 cm (Table1). The tallest genotype was EH1609-F5.B3-10 (107 cm) followed by HB-120 (103 cm) and Beka (102 cm). The shortest genotype was Miscale-21 (81 cm). Analysis of variance shows significant difference ($P < 0.0001$) among the genotypes for days to heading and days to maturity (Table 1). The difference in heading dates among tested genotypes was ranging from 59 days (HB-1533) to 71 days (HB-52). EH1609-F5.B3-10 (60 days) and EH1603-F5. B1-4 (61 days) were the second and third early genotype to head, respectively. Days to maturity ranged from 97 days (EH1609-F5.B3-10 and HB-1533) to 106 days (Beka) with the grand mean of 102 days. Early maturing genotypes complete their life cycle in relatively shorter period. Thus, early maturing genotypes have the advantage over the late in environments where rain begins late and ends early and are compatible to this type of agro ecologies. In addition, early genotypes are hunger relievers during periods of food shortages in August and September.

Table 1. Analysis of Variance for four agronomic traits

Source of Variation	Degree of freedom	Plant height (cm)	Days to heading (days)	Days to maturity (days)	Grain Yield (kg/ha)
Genotype	8	556.41**	156.78**	88.51**	100.37**
Nitrogen	1	160.5*	1.12 ^{NS}	1.38 ^{NS}	76.67 ^{NS}
Genotype × Nitrogen	8	18.95 ^{NS}	4.21 ^{NS}	2.32 ^{NS}	24.18 ^{NS}
Error	51	22.38	4.01	2.86	22.1

NS= Non Significant, * =Significant at 0.05 probability level, ** = Significant at 0.01 probability level

Table 2. Analysis of Variance for four Nitrogen Use Efficiency traits

Source of Variation	Degree of Freedom	Nitrogen Uptake Efficiency	Nitrogen Utilization Efficiency	Biomass Production Efficiency	Nitrogen Use Efficiency
Genotype	8	0.4**	27**	1220.5**	433.4**
Nitrogen	1	6.8**	14.6 ^{NS}	9.9 ^{NS}	8922.7**
Genotype× Nitrogen	8	0.08 ^{NS}	9.02 ^{NS}	132.1*	98.4 ^{NS}
Error	51	0.09	6.87	56.5	110.4

NS- Non Significant, * - Significant At 0.05 Probability Level, ** - Significant At 0.01 Probability Level

The two levels of N applied did not show considerable variation with the exception of plant height. The genotypes mean height was 96 and 93cm for the 20.5 and 41 kgha^{-1} N levels applied. This finding is in agreement with[12] who reported that plant height increased as N rate is increased in barley. This result clearly showed that it is better to apply the lower rate of nitrogen to be economical. There was no significant interaction between genotype and nitrogen levels of the agronomic traits evaluated.

3.2. Nitrogen Use Efficiency Traits

Increased use of fertilizer N in agricultural production has raised concerns, because the nitrogen surplus is at risk of leaving the plant-soil system causing environmental contamination and increased costs associated with the manufacture and distribution of N fertilizer. This has renewed research interest in increasing the efficiency use of N in different crops. Successful breeding of N efficient crop depends beside other factors on the existence of genetic variation. A genotype can be termed N efficient either, when realizing a high yield under conditions of low nitrogen supply[13] or when converting N fertilizer efficiently into yield under conditions of high nitrogen supply[14].

Highly significant variation among genotypes was observed for all the NUE traits studied (Table 2). With regard to nitrogen uptake efficiency (UPE), EH1609-F5. B3-10 (1.95 kg kg^{-1} N), HB-1533 (1.92 kg kg^{-1} N) and EH1603-F5.B1-4 (1.76 kg kg^{-1} N) were efficient in absorbing nitrogen supplied in the soil (Table 1). Inefficient genotypes were also obtained regarding uptake efficiency when compared each other. These were HB-52 (1.38 kg kg^{-1} N) and Miscale-21 (1.38 kg kg^{-1} N) (Table 3). Similar to UPE, highly significant genotypic variation was observed in the ability of the genotypes to produce grain yield from each unit of nutrient taken up (Table 2). Miscale-21 (43.12 kg kg^{-1} N), EH1609-F5.B3-10 (41.10 kg kg^{-1} N) and HB-120 (40.9 kg kg^{-1} N) were efficient genotypes in utilizing the available N in the plant (Table 3). Miscale-21 was inefficient in UPE but efficient in UTE; on the other hand, EH1609-F5.B3-10 was efficient in UPE as well as UTE. The current study clearly showed that efficient genotypes in UPE are not necessarily efficient in UTE or vice versa. As[15] reported from their long-term experiments that genetic variation exists in cereals UTE depending on genotype height. In the present study, the two genotypes, which were found efficient in UTE, were the tallest (EH1609-F5.B3-10) and the shortest (Miscale-21) with height of 106.5 and 81.3 cm, respectively.

Significant different ($P < 0.0001$) was observed among the tested genotypes in BPE (Table 1). Beka (124.78 kg kg^{-1}N), HB-52 (122.17 kg kg^{-1}N) and HB-120 (118.67 kg kg^{-1} N) produced more biomass per total above ground N.

The difference among the tested genotypes in NUE was highly significant (Table 2). Comparison of means showed that genotypes EH1609-F5.B3-10 (77.25 kg kg^{-1}N), HB-1533 (71.34 kg kg^{-1} N) and EH1603-F5.B1-4 (65.83 kg kg^{-1} N) had the highest NUE (Table 1). These efficient genotypes were also higher yielders.

Genetic variability for NUE in barley was reported[16]. As[17] selected some nitrogen efficient genotypes by studying on 40 barley cultivars. The differences in UPE, UTE and NUE of the genotypes studied may be due to several factors.

According to[18], these factors may include root morphology, extension, biochemical, and physiological mechanisms in nitrate assimilation and use. Efficiency of N uptake and N utilization in the production of grain requires that those processes associated with absorption, translocation, assimilation and redistribution of N operate efficiently[5].

Highly significant variation was observed among the two levels of N applied for all the traits studied except UTE and

BPE which were non-significant. For UPE, Genotypes absorbed more N at 20.5 kg N ha^{-1} (1.91 kg kg^{-1} N) than at 41 kg ha N^{-1} (1.30 kg kg^{-1} N) which is in agreement with[19] who reported that UPE is an important component of NUE under low N conditions. EH1609-F5.B3-10, EH1603-F5. B1-4 and HB-1533 had higher UPE at 20.5 kg N ha^{-1} with the values of 2.31, 2.28 and 2.14, respectively. Lack of genotype with N application level interactions for UPE studied indicated that genotypes responded similarly to the two N application levels.

For NUE, respective values of 74.11 and 51.85 kg kg^{-1} N at 20.5 kg ha^{-1} and 41 kg ha^{-1} N levels were observed. The finding of this research is in agreement with[20] which states that NUE declined substantially as soil available N increased. As[19] reported similar trends. Nitrogen use efficiency is comprises of uptake and utilization efficiency. As it was discussed earlier, significant and non-significant difference was obtained by the two levels of N applied for uptake and utilization efficiency respectively. Therefore, the cause for the decline in NUE as the level of N increased is because of a decline in uptake efficiency.

With regard to interaction, all the studied traits were non-significant except biomass production efficiency (BPE) which was significant.EH1603-F5.B1-4 (98.48) and HB-120 (125.29) produced high BPE both at the higher rate of N application while Miscale-21 (110.29) produced high biomass at the lower level of N application.

The lack of significant difference due to the interaction of genotype by N level in this investigation indicates that genotypes responded similarly to the two N application levels. High NUE values are obtained for all genotypes at lower level of N level applied. So, in the absence of interaction efficient genotypes can be selected at the lower level of N applied. In general, presence or absence of interactions strongly depends upon the behavior of the genotypes.

Table 3. Grand mean, Mean, LSD and CV values of four NUE traits

ENTRY	Nitrogen Uptake Efficiency	Nitrogen Utilization Efficiency	Biomass Production Efficiency	Nitrogen Use Efficiency
Genotypes				
Arna	1.47	39.18	114.86	57.34
Beka	1.57	37.84	124.78	59.6
EH1603-F5.B1-4	1.76	37.95	92.32	65.83
EH1609-F5.B3-10	1.95	41.10	97.98	77.25
HB-120	1.52	40.90	118.67	62.18
HB-1533	1.92	37.58	93.66	71.34
HB-52	1.38	39.34	122.17	53.63
Holker	1.52	39.03	112.39	59.42
Miscale-21	1.38	43.12	104.12	60.21
SE	0.13	0.94	3.06	4.58
N-Label				
N1	1.3	40.01	109.37	51.84
N2	1.92	39.11	108.62	74.11
Grand Mean	1.61	39.56	108.99	62.98
LSD	0.432	3.71	10.63	14.86
CV (%)	18.97	6.63	6.89	16.68

SE= Standard Error of the Mean, LSD-=Least Significant Difference, CV=Coefficient of Variation

Table 4. Variances, Coefficient of variations and Heritability of studied traits

Trait	σ^2g	σ^2n	σ^2gn	σ^2e	σ^2p	PCV (%)	GCV (%)	NCV (%)	GNCV (%)	H (%)
Plant Height	67	3.9	0.8	22	94	10	8.7	2.1	0.98	71
Grain Yield	9.5	1.5	0.5	22	34	19.6	10.4	4.07	2.43	28
Days to Heading	19	0.1	3.2	4.0	26	8	6.76	0.44	2.77	72
Days to Maturity	11	0.02	0.13	2.9	14	3.6	3.21	0.14	0.35	78
Nitrogen Uptake Efficiency	0.04	0.2	0.002	0.1	0.3	35	11.8	26.9	3.1	12
Nitrogen Utilisation Efficiency	2.2	0.2	0.53	6.9	9.8	8	3.75	0.97	1.83	23
Nitrogen Use Efficiency	42	245	3	110	400	32	10.2	24.7	2.73	10
Biomass Production Efficiency	136	0.05	18.9	56	211.	13	10.7	0.2	4	64

σ^2g = Genotypic variance, σ^2n = Variance due to N - level, σ^2gn = Variance due to interaction (g×n), σ^2e = Environmental variance, σ^2p = Phenotypic variance, PCV = Phenotypic Coefficient of Variation, GCV = Genotypic Coefficient of Variation, NCV = Coefficient of Variation due to N- level, NGCV = Coefficient of Variation due to interaction, H = Heritability

3.3. Estimation of Genetic Parameters

The genotypic and phenotypic variance components and coefficients of phenotypic and genotypic variability for all the traits studied were calculated (Table 4). An effective breeding program largely depends upon genetic variability. Thus, success of genetic improvement is attributed to the magnitude and nature of variability present for a specific character. The polygenic variation may be phenotypic, genotypic or environmental. According to Deshmukh et al.[21] phenotypic and genotypic values greater than 20% are regarded as high, whereas values less than 10% are considered low and values between 10% and 20% as medium.

High phenotypic coefficient of variation (PCV) was observed for UPE (34.6 %) and NUE (31.6 %). Medium PCV was observed for plant height, grain yield, and BPE, but the remaining traits studied showed low PCV. Higher PCV value indicated high environmental effect on the traits in this study. High genotypic coefficient of variation (GCV) was not observed for the studied traits. Medium GCV was observed for grain yield, NUE and BPE. Low GCV was observed for the remaining traits. High GCV value of characters suggested the possibility of improving these traits through selection**.** High nitrogen coefficient of variation (NCV) was viewed for UPE (26.9%) and NUE (24.7%). These values show that there is a room for direct selection of the nitrogen level of the two levels applied for the above traits. If we take UPE for instance, genotypes receiving 20.5 kg N ha^{-1} were more efficient in absorbing nitrogen from the soil than genotypes receiving 41 kg N ha^{-1}. Medium NCV was not observed for the studied traits. Low NCV was observed for the remaining traits. Coefficient of variation due to interaction of genotype with N level (GNCV) was low for all the traits studied which gives no room for selecting genotypes and nitrogen levels simultaneously.

3.4. Estimation of Broad sense-Heritability

In this study estimate of heritability in broad sense ranged from 10% for NUE to 78% for days to maturity (DMA). According to Singh[22] if heritability of a character is very high, say 80% or more, selection for such characters could be easy. This is because there would be a close correspondence between the genotype and the phenotype due to the relative small contribution of the environment to the phenotype. Nevertheless, for characters with low heritability, say 40% or less, selection may be considerably difficult or virtually impractical due to the masking effect of the environment. Heritability is moderate when it is between 40 and 80%.

High heritability was not observed for the studied traits. Traits showing moderate heritability were plant height (71%), days to heading (72%) and days to maturity (78%). Those traits that showed high and moderate heritability are found to have high GCV value than traits that showed low heritability. Selection for these traits is relatively easy because most of the variation is genetic rather than environmental. On the other hand, traits with high PCV have less heritability (Table 4) which means variation for these traits is more of environmental rather than genetic and it is not advisable to select for these traits. The NUE trait showing moderate heritability was BPE (64%). Of the traits of malting barley studied in this experiment, 50% show low heritability including grain yield (28%) which makes selecting for these traits difficult because environmental effect is more evident that genetic effect.

4. Conclusions and Recommendations

The presence of highly significant variation for NUE and yield indicates that efficient and high yielding malting barley genotypes can be directly selected. Genotypes EH1609-F5. B3-10, EH1603-F5.B1-4 and HB-1533 were efficient in N use and are higher yielder as compared to other genotypes.

The two levels of nitrogen applied did not show significant difference for the majority of the traits studied. Therefore to be economical as well as to prevent environmental effect of N, it is better to use the lower level of N. High phenotypic coefficient of variation (PCV) was observed for UPE (34.6%) and NUE (31.6%). High genotypic coefficient of variation (GCV) was not observed for the studied traits. Generally choosing efficient genotypes and the application of low levels of N seems to be the most efficient way to supply nitrogen when grain yield, NUE, and quality are our goals.

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