Genotype-by-Environment Interaction for Quantitative Trait Loci Affecting Nitrogen Use Efficiency and Associated Traits in Potato

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Abstract
Deciphering the genetic basis of complex traits like nitrogen use efficiency (NUE) requires understanding the genotype-by-environment (G × E) interaction and linking physiological functions and agronomic traits to DNA markers. Multi-environment experiments were conducted in different environments representing low and high nitrogen levels combined with rain-fed and irrigation production conditions at three different locations in Ethiopia: Debre-Tabor, Injibara and Koga, in 2013 and 2015. The objectives of the study were to determine the G × E interaction and stability of genotypes for NUE of potato and to identify markers associated with NUE and NUE-related agronomic and physiological traits in potato under these diverse environments. Data were analyzed using GenStat, and genotype plus the genotype and environment (GGE) biplot model; the marker-trait associations were discovered using the R-software package GWASpoly. The analysis of variance that included location and production system had estimates for genotype variance (σ^2_g) that were low compared with the estimates for environment variance (σ^2_e) and genotype-by-environment interaction variance (σ^2_ge) for most measured traits at both N levels. The GGE analysis identified two mega-environments that coincided with the two production systems. The high N level environments both at Debre-Tabor and Injibara, and the low N environment at Koga, respectively, were the most suitable environments under these diverse environments.
for discriminating the potato cultivars and being representative test environments for NUE evaluation in the rain-fed mega-environment and irrigation mega-environment. A total of 77 marker trait associations were identified for NUE and NUE-related agronomic and physiological traits. Multi-trait genomic regions that harbored significant marker-trait associations for NUE and NUE-related traits were found on chromosomes III, V and VI. The effect of production season was greater than the effect of N levels on QTL × environment interaction for most NUE-related traits.

Keywords Genotype-by-environment interaction · Mega-environment · Nitrogen use efficiency · Potato · Quantitative trait loci

Introduction

Nitrogen (N) is one of the most essential agricultural inputs for high crop production and productivity in the world. Application of mineral N fertilizer is the main driver for restoring soil N and high crop yields (Hirel et al. 2011). A dramatic increase has occurred in crop yield through global utilization of synthetic N; however, in the last decades, the consumption rate of N fertilizer and the crop yield increase were no longer proportional. In the last 40 years, the amount of mineral N application to cultivated crops increased 7.4-fold, while the overall crop yield increase was only 2.4-fold (Tilman et al. 2002). Potato is one of the N fertilizer–demanding crops, requiring 180–240 kg N/ha to produce high tuber yields (35–45 t/ha); of total applied N, plants acquire only 40–50% and the remaining N is lost in the environment (Trehan and Singh 2013). Increasing the N fertilizer application rate reduced the average net return of potato production. Zotarelli et al. (2021) reported that net average return reduced by 4.3% as the N fertilizer application rate increased from 280 to 336 kg/ha. The financial cost and waste of the inefficient fertilizer technologies particularly burden smallholder farmers in developing countries. Moreover, the excessive loss of nitrate from the potato root zone results in nitrate contamination of groundwater. Elevated ground water nitrate concentrations have been attributed to commercial potato production (Hill 1986; Richards et al. 1990) and significant emissions of nitrous oxide (a greenhouse gas) have been measured from potato fields (Flessa et al. 2002; Burton et al. 2008).

Thus, the recent increase of N fertilizer use has not only led to minor yield gains and reduced nitrogen use efficiency of the crops, but also to serious environmental problems (Cassman et al. 2003; David et al. 2010). Considering the crop yield trends together with the forecasted population growth and environmental strain caused by synthetic N fertilizer use, breeding efforts need to focus on traits related to yield stability, maintenance with suboptimal N availability and nitrogen use efficiency (NUE). NUE was defined by different authors in different ways, depending on the objective of the study and the crop under study. For example, Moll et al. (1982) defined NUE as the yield per unit of nitrogen resource available to the plant. Bock (1984) defined NUE as the total dry weight of the plant per unit of soil N. In this study, we follow the definition of Moll et al. (1982). Breeding for N-efficient crop cultivars is a sustainable strategy towards this goal. However, NUE is a complex trait controlled by a large number of genes and highly affected by environmental variation. Deciphering the genetic basis of complex traits like NUE requires the linking of physiological functions.
and agronomic traits to DNA markers (Prioul et al. 1997; Hirel et al. 2007). Knowledge about the genes and molecular mechanisms involved in N metabolism and the traits that are affected by low N input is important to elucidate the genetic basis of potato NUE and discover the genes underlying these traits.

In potato, a wide range of variation in NUE has been reported in cultivated clonal selections, and accessions of wild species (Errebhi et al. 1998; Errebhi et al. 1999; Zvomuya et al. 2002; Zebarth et al. 2004; Sharifi et al. 2007; Tiemens-Hulscher et al. 2012; Ospina et al. 2014; Baye et al. 2020a) suggesting the possibility of improving NUE through breeding. Various traits related to NUE and contributing to NUE were used to increase the efficiency of the selection process and support the development of cultivars that give reasonable yield under low N availability (Errebhi et al. 1998). Among these traits, nitrogen uptake efficiency, yield and its components, Leaf Area Index (LAI) and period of maximum soil covering showed significant variation at low N conditions (Tiemens-Hulscher et al. 2012). Significant variation for N use efficiency and its components (N utilization efficiency (NUtE) and N uptake efficiency (NUpE) has been reported for fresh market red potato varieties evaluated under low and high N conditions (Jones et al. 2019). According to Jones and his colleagues, NUtE showed strong correlation with NUE and tuber yield under low N condition, whereas NUpE showed strong correlation with tuber yield and NUE at high N condition. In breeding of new potato varieties with better NUE, genotype-dependent nitrogen use efficiency studies are relevant (Kollaricsné Horváth et al. 2019).

Still the genetic base of NUE in potato is poorly understood, and the complexity of many phenotypic traits involved in adaptation to stress conditions is likely to arise from a number of quantitative trait loci (QTLs). QTL analysis with a wide range of molecular markers provides opportunities not only for the identification of QTLs that determine the phenotypic value of a particular trait, but also for the analysis of the relationships between traits (Lebreton et al. 1995; Simko et al. 1997). Especially, the Genome Wide Association analysis has the potential to detect QTLs associated with the desired trait and, when a large number of markers are available for a large set of genotypes even to identify the causal polymorphisms within a gene that are responsible for the difference in two alternate phenotypes (Gupta et al. 2005). However, as the performance of genotypes is affected by the environment, the expression of QTLs can be affected by environmental variations. Some QTLs exist consistently over environments (constitutive QTLs), while other QTLs are identified only in specific environments, or modulate their effect with changing environmental conditions (adaptive QTLs) (Tuberosa et al. 2008). Studies of QTLs affecting traits related to NUE have been reported in maize (Agrama et al. 1999; Hirel and Lea 2001; Li et al. 2015; Mandolino et al. 2018), Arabidopsis (Loudet et al. 2003) and rice (Cho et al. 2007; Zhou et al. 2017; Vishnukiran et al. 2020), and many of these QTLs were dependent on N levels.

In potato, QTL studies for NUE are still very limited. Only recently, QTLs affecting traits related to NUE under contrasting N regimes were reported (Baye Getahun et al. 2020b; Ospina et al. 2021). Moreover, major nitrogen-responsive genes such as nitrate-reductase, nitrite-reductase, ammonium transporters and asparagine synthase genes have been identified in different potato genotypes under different N levels (Kollaricsné Horváth et al. 2019).

The objectives of the current study were as follows: (i) to evaluate the influence of locations, N levels and production conditions on NUE and on yield stability of potato
cultivars; (ii) to identify QTLs/markers associated with NUE and NUE-related agronomic and physiological traits; and (iii) to assess the QTLs-by-Environment interaction and stability of QTLs, which may help in further identification of candidate genes that may be useful for allele mining in potato germplasm, and marker-assisted selection for NUE improvement of potato.

**Materials and Methods**

A set of 88 tetraploid potato cultivars and progenitor lines were used for G × E and association mapping experiments of this study (Supplementary Table 1). The materials have been released for commercial production in Europe with release dates ranging from 1908 to 2013 (Berloo et al. 2007). The panel represents European and American origins of potato cultivars with different market niches and phenotypic diversity for agronomic traits. Based on their market niche, the association panel set includes 51 fresh consumption, 12 general purposes, and 23 processing industry, 1 starch and 1 old cultivar. The seeds were shipped from Europe to Ethiopia at early sprouting stage, and transport took time which was a variable but which was similar to all sites. So the seed tubers within the same production season had no physiological age difference. The materials were kindly provided by the Dutch potato–breeding company HZPC Holland BV.

**Description of Experimental Areas**

The experiments were conducted at three different locations in northwestern Ethiopia: at Injibara and Debre-Tabor in 2013 under rainfed conditions and at Koga and Injibara in 2015 in the dry season under irrigation. The locations are situated in the Amhara region (Figure 1), which is the major potato-growing area of the country accounting for

![Fig. 1 Location map of the study area](image-url)
about 40% of the Ethiopian potato farmers (CSA, 2008/2009). Debre-Tabor and Injibara are located at higher altitudes. Debre-Tabor is located at 2650 masl with an expected average annual rainfall of 1500 mm, and Injibara is located 2600 masl with 2300 mm annual average rainfall. The average rainfall provided in Table 1 is the average of the two growing seasons (2013 and 2015) for the specific environments. For example, at Debre-Tabor in 2013, it was 1600 mm and in 2014 it was 1400 mm and the average of the two seasons was 1500 mm; similarly, at Injibara, it was 2350 mm in 2013 and 2250 mm in 2015 and the average of the two seasons was 2300 mm. As their annual average rainfall shows, Debre-Tabor is relatively dry highland, while Injibara is a wet highland area. The trials were carried out during the main rainfed production season. The main meher rainfed production season begins in May and ends in September, and the rainfall distribution in 2013 was good for potato production in both locations, although its intensity was high.

The irrigation experiments in 2015 were conducted during the dry season (February to June). The irrigation period of the year in Ethiopia is characterized by dry weather, high day temperature, relatively low night temperature and low disease incidence. The two experimental sites are located in different agroecological zones. Koga is located at mid altitude (1900 masl), while Injibara is located at higher altitude (2600 masl). Irrigation water was applied every week in both locations using furrow irrigation. Although the amount of applied irrigation water was not quantified because of shortage of irrigation equipment, the furrow dimensions were similar to have as much uniform distribution across the experimental fields as possible at both locations. There was no scarcity of irrigation water at Koga, but at Injibara, there was scarcity, particularly at the beginning of the experiment. The environmental variation experienced from location to location even within a short distance, and from rainfed production season to irrigation production season is among the most dominant features of the Ethiopian environmental conditions (EMA 1988). We defined eight target environments for the data analysis. Each location combined with a production season and N level was considered as a separate target environment making a total of eight test environments for this study. Description of the test locations and the eight defined environments are presented in Table 1 and Table 2, respectively.

Table 1 Geographical positions of the experimental locations with average rainfall and minimum (Min) and maximum (Max) temperature in the experimental seasons (2013 and 2015) and soil type and soil pH

| Geographical position | Temperature (°C) | Soil type | Soil pH |
|-----------------------|------------------|-----------|---------|
| Locations | Latitude | Longitude | Altitude (m asl) | Average rainfall (mm) | Min | Max |
| Debre-Tabor | 11.89 °N | 38.04 °E | 2650 | 1500 | 11.8 | 23 | luvisol | 5.2 |
| Injibara | 10.85 °N | 36.80 °E | 2600 | 2300 | 8.0 | 22 | acrisol | 4.8 |
| Koga | 11.37 °N | 37.12 °E | 1900 | 1400 | 9.0 | 32 | nitosol | 5.4 |
Field Trials and Experimental Design

The field experiments were conducted with a similar experimental set-up in all locations under rainfed and irrigation production conditions. The trials had a split-plot design with two replications, where the main plots were assigned to the low and high N rates (40 kg/ha and 120 kg/ha) and the sub-plots to the genotypes. Each experimental plot consisted of 10 tubers planted in a row with intra-row spacing of 0.30 m. Genotypes were 0.75 m spaced, and each experimental plot was bordered by a reference potato cultivar (Gudenie). Application of irrigation water, pest and disease management, weeding and ridging and other cultivations were done following the recommendation of each location and when required. The total amounts of N (40 and 120 kg/ha) included the available N in the soil and application of commercial N fertilizer in the form of urea and di-ammonium phosphate (DAP). Phosphorus fertilizer was applied following the recommendation for each location in the form of DAP and tri-supper phosphate (TSP). The whole P source was applied at planting while N application was split into two: a week after emergence and at the start of flowering.

Phenotypic Measurements

The phenotypic measurements were carried out in similar manner at all experimental locations (Koga, Debre-Tabor and Injibara). Plant height (PH), chlorophyll content (CC) at lower and upper part of the canopy using aSPAD-502 chlorophyll meter (Minolta Co. Ltd. Japan) were measured when 50% of the genotypes in a plot had flowers. The readings for chlorophyll content were taken on the third or fourth leaf from the top of the plant for upper leaf chlorophyll content (UCC), and the second or the third leaf from the base of the plant for lower leaf chlorophyll content (LCC). Stem number per plant (SNPP) was counted before the plant foliage declined. Canopy cover dynamics or soil cover (SC) was assessed every 5 days starting from date of full emergence to the declining phase of the crop growth using a 0.6 m × 0.75 m frame with 100 grid squares, positioned over the same middle plants in a plot for each
measurement. The beta thermal time for canopy cover assessment was calculated from the date of emergence for each experimental unit using the sigmoid part of the beta function for determinate growth (Yin et al. 2003), and estimated cardinal temperatures (5.5°C as a base temperature, 23.37°C as optimum and 34.58°C as ceiling temperature) that determine the vegetative growth of potato (Khan et al. 2013; Khan et al. 2019). Hourly temperature data was collected from the nearest weather station for each location. The model for canopy development was fitted using the soil cover data, beta thermal time for each assessment and the canopy cover measurements, and the following model parameters were estimated using the NOLIN procedure of SAS, SAS Institute Inc, 2004: the inflection point in the build-up phase of the growth curve (tm1; in thermal days, td), time at which canopy cover reaches its maximum (t1; td), the maximum canopy cover value with percentage of soil cover as unit (Vmax; %), time for onset of canopy decline (t2; td), time when canopy cover reaches zero (te; td) and area under the curve for the entire crop growth cycle (AUC; in % td) (Khan et al. 2013).

Days to maturity (DTM), determined as the number of the days from emergence to the day at which more than 90% of the plants in a plot attained physiological maturity (90% of the haulm tissues brown) was assessed every day starting from the time that early varieties showed the first signs of leaf yellowing.

Tuber traits: tuber number per plant (TNPP), average tuber weight (ATW), tuber yield per plant (TYPP), specific gravity (SG), tuber dry matter percentage (%TDM) and nitrogen use efficiency (NUE; defined as dry tuber weight per unit N available (N applied + N available in the soil) were measured and estimated at harvest. Specific gravity (SG) was determined using the tuber-specific gravity procedure of weight in air and under water (Murphy and Goven 1959). In evaluating the SG of each variety, healthy and marketable-sized grade (20 mm and above) tubers were selected randomly from each variety harvest. Then, tubers were cleaned, and weighed both in air and in water following the procedure of Murphy and Goven (1959). Specific gravity values were computed using the following formula:

\[ SG = \frac{W_1}{W_1 - W_2} \]

where SG is the specific gravity of the material, \( W_1 \) is the weight in air of the sample tuber, in grams, and \( W_2 \) is the weight of the sample completely immersed in water, in grams.

Tuber dry matter content (TDM%) normally is determined as a ratio of dry tuber weight to fresh weight expressed in percentage; we determined TDM% indirectly from SG using empirical conversion factors following the equation of Kleinkopf et al. (1987): solid (dry matter %) = \(-214.9206 + (218.1852 \times SG)\). Tuber dry weight (TDW) was estimated indirectly from specific gravity and tuber dry matter content in percent, using the following formula:

\[ DW_t = \frac{TDM \times FW_t}{100} \]

Where DWt is the dry weight of the tuber in grams; TDM is the tuber dry matter content (%); FWt is the fresh weight of the tuber in grams.
Nitrogen use efficiency (NUE) is calculated as the yield per unit of N resource available to the plant (Moll et al. 1982). In this study, NUE was determined as the tuber dry matter production, or dry weight of the tubers/ha, per unit of N supplied/ha (N in the soil + applied N).

Data Analysis

The analysis of variance of NUE data for each trial was done using a split-plot design with GenStat 18th edition software. The adjusted means of each trial were used in a combined analysis of variance to evaluate the main effect of environment (E), genotype (G), genotype-by-environment interaction (GE) variances, and to find estimates of the genotypic variance ($\sigma^2_g$), Genotype-by-Environment interactions ($\sigma^2_{ge}$) variance and environmental variance ($\sigma^2_e$) for each trait at each N level. Subsequently, these estimates were used to calculate the heritability ($H^2$) of each trait based on genotype means over the four low N and four high N level environments using the formula:

$$H^2 = \frac{\sigma^2_g}{\sigma^2_g + (\sigma^2_{ge}/e) + (\sigma^2_e/re)}$$

where $e$ and $r$ are the number of environments and replications per environment, respectively. The best linear unbiased estimates (BLUEs) were computed to generate phenotypic values for marker trait association studies using breeding view, the IBP Breeding Management System (BMS) version 3.0.9 (https://www.integratedbreeding.net/breeding-management-systemBMS-breeding) software. Each N level at each location in each production season was considered as a single environment for the association mapping analysis with a total of eight environments.

Further partitioning and analysis of G × E interaction was carried out with GGE biplot model using GGE software (Yan 2001). The GGE biplot was constructed using the first two principal components (PC1 and PC2) derived from environment-centered NUE data (Yan et al. 2000). Environment-centered data is the data with the grand mean and the environmental effects removed from the data as defined by the following GGE model:

$$Y_{ij} - \mu - \beta_j = \lambda_1\xi_{i1}\eta_{1j} + \lambda_2\xi_{i2}\eta_{2j} + \varepsilon_{ij}$$

where $Y_{ij}$ is the measured mean of genotype $i$ ($=1,2,...,n$) in environment $j$ ($=1,2,...,m$); $\mu$ is the grand mean; $\beta_j$ is the main effect of environment $j$; $\lambda_1$ and $\lambda_2$ are the singular values (SV) for the first and second principal component (PC1 and PC2), respectively; $\xi_{i1}$ and $\xi_{i2}$ are eigenvectors of genotype $i$ for PC1 and PC2, respectively; $\eta_{1j}$ and $\eta_{2j}$ are eigenvectors of environment $j$ for PC1 and PC2, respectively; $\varepsilon_{ij}$ is the residual associated with genotype $i$ in environment $j$.

Genotypic Data Analyses

The panel was genotyped with a 20k Infinium SNP array (Vos et al. 2015). A total of 14,587 markers were scored in 5 dosage classes (nulliplex, simplex, duplex, triplex and...
tetraplex) depending on the number of copies of the allele (0 to 4) using fitTetra (Voorrips et al. 2011). Of the markers scored, 12,519 were polymorphic SNPs that were used for genome-wide association mapping. Linkage disequilibrium (LD) and population structure were analyzed previously for a larger genotype set by d’Hoop et al. (2010) that included 70 cultivars used for this study. All individual environments as well as combined mean data (all low N environments taken together and all high N environments) for each trait were subjected to single marker trait association analysis using the R software package for autotetraploids (GWASpoly). This package is unique in its ability to conduct the single marker test for association using different models of gene action (Rosyara et al. 2016). GWASpoly used mixed model analysis to perform marker trait association analysis, and for each trait, four GWAS were conducted based on additive, simplex-dominant, duplex-dominant and general genetic models. The package used both a Q and a K matrix; the Q-matrix to account for population structure, and the K-matrix to correct for kinship of the association panel, to reduce the plausible but false marker-trait associations. Both results (Linkage disequilibrium (LD) and Q-matrix) revealed that there was no clear structure in the panel, suggesting that the population was suitable for association mapping studies. Bonferroni correction for a genome wide-scan was used as a QTL detection threshold at 5% significance level. When multiple significant markers were detected within a 10-Mb region, only the most significant marker was reported along with the corresponding genetic model.

**Results**

**Variance Analysis**

The individual and combined analysis of variance for tuber yield revealed highly significant differences ($P \leq 0.001$) among genotypes in all environments except in E8 (Koga, high N in 2015) (Table 3). The experimental coefficients of variation (CV) were relatively low, ranging from 15 to 25%, except in E8 (CV 36%). The irrigation production season test environments (E5, E6, E7 and E8) showed higher tuber yield and NUE performance compared with rainfed production season test environments.

**Table 3** Mean squares of individual analysis of variance by environment for tuber yield for potato genotypes in eight environments

| Source of variation | df | E1   | E2   | E3   | E4   | E5   | E6   | E7   | E8   |
|---------------------|----|------|------|------|------|------|------|------|------|
| Replication         |    | 0.4  | 0.11 | 0.014| 0.28 | 0.075| 0.23 | 0.45 | 0.13 |
| Genotype            | 80 | 0.01***| 0.02***| 0.003***| 0.014***| 0.014***| 0.021***| 0.045***| 0.12 |
| Residual            | 80 | 0.003 | 0.005 | 0.001 | 0.004 | 0.004 | 0.005 | 0.025 | 0.09 |
| Mean (kg/plant)     |    | 0.31 | 0.44 | 0.22 | 0.43 | 0.35 | 0.42 | 0.63 | 0.89 |
| CV (%)              |    | 17.6 | 16.4 | 16.5 | 14.5 | 17.7 | 17.1 | 25.4 | 35.6 |

***significant at 0.001. CV (%) coefficient of variation in percentage.
(E1, E2, E3 and E4) (Tables 3 and 4). However, within the irrigation production season, test environments at Injibara (E5 and E6) showed lower performance compared with test environments at Koga (E7 and E8). The low tuber yield and NUE performance of the cultivars at Injibara in the irrigation production season may be attributed to shortage of irrigation water especially at early (emergence and growth) stages of the crop. Genotype G61 “Navigator” had the highest tuber yield (0.6 kg/plant). All other genotypes yielded 0.35 kg or more except G6 “Annabelle” (0.32 kg/plant). Among the environments, E8 had the highest mean yield (0.89 kg/plant) (Supplementary Table 2).

The individual analysis of variance for NUE was significantly different ($P \leq 0.05$) among genotypes in E8 and highly significantly different ($P \leq 0.001$) in other environments (Table 4). The coefficients of variations (CV) were a bit higher in irrigation experiments compared with rainfed; this higher CV might have been related with irrigation water management. As the experiments were conducted using furrow irrigation at field conditions, there might have been differences in seepage and other factors between plots that might have caused higher CV values. The NUE was higher at low N than at high N conditions; the reason might have been that at low N, there might not have been extra N available for loss as compared with high N, i.e. at low N condition, the amount of applied N might have been more utilized by the plant. At high N, there might have been extra N which was vulnerable to loss due to various factors such as leaching, volatility and others; due to these reasons, NUE might have been low at high N.

The combined analysis of variance over environments revealed that potato NUE was significantly ($P<0.001$) affected by environment (E), genotype (G) and genotype-by-environment interaction (G×E) (Table 5). The environment accounted for 79.6% of the total sum of squares (SS) of (G+E +G×E) variation, which was the largest contribution to the total variation. The genotype and genotype-by-environment interaction, respectively, accounted for only 4.1% and 16.3% of the total sum of square variation (Table 5). The significant effect of the G×E interaction in the combined analysis of variance suggested that the genotypes had variable performance in the tested environments (different best performers at different environments). The contribution of the environment to the total variation was high compared with the effect of G × E and G. This high environmental variance compared with genotypic variance, particularly in the

### Table 4
Mean squares of individual analysis of variance by environment for nitrogen use efficiency for potato genotypes in eight environments

| Source of variation | df | E1   | E2   | E3   | E4   | E5   | E6   | E7   | E8   |
|---------------------|----|------|------|------|------|------|------|------|------|
| Replication         |    | 453.9| 12.69| 554.2| 301.61| 1323.6| 479.27| 18974| 5454.4|
| Genotype            | 80 | 243.43***| 38.98**| 430.9***| 64.10***| 438.2***| 83.68***| 1454.3***| 416.8* |
| Residual            | 80 | 89.39| 20.04| 102.6| 21.64| 194.4| 34.94| 471.5| 288.2 |
| mean (kg/kg)        | 37.06| 14.60| 41.21| 15.64| 41.21| 15.63| 101.31| 51.11 |
| CV (%)              | 25.5| 30.7| 24.6| 29.7| 35.4| 37.3| 21.4| 33.2 |

*significant at 0.05, **significant at 0.01, ***significant at 0.001. CV (%) coefficient of variation in percentage
irrigation production system, might have been attributed to irrigation management. As we conducted the field experiment using furrow irrigation, seepage and other factors might have enhanced within and between field variations, and thus increased the environmental variance in the irrigation experiments.

Mega Environment Analysis

Considering the mega-environment components of a target region for a specific crop is a precondition for determining proper approaches of genotype evaluation and cultivar recommendation. The vector view of the GGE biplot for NUE of 81 potato cultivars evaluated in 8 environments is shown in Figure 2. The environments are connected to the biplot origin by the vectors. The percentages of GGE explained by PC1 and PC2 were 31.4% and 25.8%, respectively, and the biplot explained 57.2% of the total variation due to G and GE using an environment-standardized model. In this case, the total variation is referred to as the variation due to the G and G×E, because the variation due to the environment main effect was excluded by this model. The GGE and especially PC1 clearly separated the two production seasons (irrigation and rainfed). All environments within the same production season appeared to be correlated (as indicated by the less than 90° angle between them), while the two production seasons appeared to be negatively correlated (as indicated by more than 90° angles) (Yan 2002). The negative correlations between groups of test environments are a strong indication that the environments can be grouped in two different mega-environments.

Mega-environments can also be defined by which-won-where patterns, and the GGE biplot is an effective graph to show these in a genotype-by-environment dataset. The biplot in Figure 2b is similar to the biplot Figure 2a except that the environment vectors were removed and a polygon with lines perpendicular to the polygon sides was added. The polygon was drawn based on cultivars placed away from the biplot origin so that all cultivars were included in the polygon. The eight environments fell into two sectors delineated by the straight lines radiating from the biplot origin and perpendicular to sides of the polygon.

Table 5 Combined analysis of variance for NUE (kg/kg) of potato cultivars for all eight environments considered together

| Source of variation | DF   | SS     | MS    | V     | P       | Variation (%) |
|---------------------|------|--------|-------|-------|---------|---------------|
| Environment (E)     | 7    | 901752.8 | 128821.8 | 718.35 | ≤ 0.001 | 79.6          |
| Genotype (G)        | 80   | 46419.3 | 580.2  | 3.24  | ≤ 0.001 | 4.1           |
| GE                  | 560  | 184643.6 | 329.7  | 1.84  | ≤ 0.001 | 16.3          |
| Residual            | 647  | 116026.9 | 179.3  |       |         |               |
| Total               | 1295 | 1250565 |       |       |         |               |
| Mean                |      | 40.1   |       |       |         |               |
| CV (%)              |      | 33.4   |       |       |         |               |

DF degrees of freedom, SS sum of squares, MS mean of square, V variance, GE genotype-by-environment interaction, CV coefficient of variation, P significance level
The sector delineated by lines 1 and 7 comprises the four rainfed environments E1, E2, E3 and E4. The genotype G43 (Kuras) is placed on the vertex of the polygon for this sector, and is therefore considered the most nitrogen efficient cultivar for this group.
of environments (see also Suppl. Table 3). The second sector is defined by the radiate lines 6 and 7, and includes the four environments of the irrigation production season (E5, E6, E7 and E8). Genotype G3 is on the vertex for this section suggesting that G3 (Agria) was the winner at these environments, and this genotype indeed had the highest NUE in the irrigation production season trials. Thus, we identified two mega-environments (the irrigation and rainfed), and the test environment evaluation was done separately for each of the two mega environments.

**Test Environment Evaluation**

Evaluating the test environments is helpful to identify test environments that can effectively identify superior genotypes and representative test environments for a mega environment, in our case for the rain-fed and the irrigated production season. The GGE biplots in Figures 3 and 4 were constructed based on environment-focused singular value partitioning for the two production seasons separately, in order to visualize the representativeness and genotype-discriminating power of the test environments in these mega environments. In the two biplots, the single-arrowed red line that passes through the biplot origin is the average environment axis (AEA), and the small red circle on the AEA represents the average environment. The double-arrowed blue line perpendicular to the AEA represents the average environment coordination (AEC). The representativeness of the environments is measured by the cosine of the angle between the test environment and AEA; the closer the test environment is to the average environment,
the more desirable it is as core testing environment (Yan et al. 2007). The vector length from the origin of the biplot to the environments is a measure of the discriminating power of the environments. Figure 3 demonstrates that all test environments are positively correlated with the AEA, and therefore they were all representative but to different degrees. E2 and E4 (the high N environments) were closest to the average environment with good discriminating ability (long vector lengths) suggesting that these are the test environments that best represent the rainfed mega environment, while E1 and E3 (the low N environments) were well-discriminating but less representative environments.

Similar to the rainfed mega environment, all four test environments in the irrigation mega-environment were correlated with the AEA of the mega environment (Figure 4). E7 was more representative, while E8 was less representative. The two testing environments at Injibara (E5 and E6, low and high N) were strongly correlated with each other, indicating their similarity in discriminating the genotypes and representativeness of the mega-environment.

Estimates of Variance Components and Heritability

The analysis of variance that included location and production system had estimates for genotype variance ($\sigma^2_g$) that were low compared with the estimates for environment variance ($\sigma^2_e$) and genotype-by-environment interaction variance ($\sigma^2_{ge}$) for most measured traits at both N levels (Table 6). The varieties showed rapid initial canopy development and matured early; however, the canopy development did not reach to maximum stage (below 100%). The average days to maturity were 73 and 74 days at low and high N, respectively. The average canopy growth ($V_{max}$) was recorded as
45.4% and 61.5% at low and high N condition, respectively. This early maturity and low canopy development may be attributed to environmental factors such as photoperiod and temperature, as the potato varieties were tested in tropical conditions. Overall, the canopy growth and canopy-related traits such as tm1, t1, t2, te, t2-t1, Vmax, AP1, AP2, AP3 and AUC were highly affected by the environment. This is reflected in the heritability estimates: almost all heritability estimates of these canopy cover related traits were low. Traits that showed a higher estimate of genotypic variance than environment at each location and production system also showed high genotypic variance in the analysis that compared production systems (with data from locations within a production system combined) (Supplementary Table 4).

The association panel included three distinct market groups (fresh consumption type, general purpose, and processing type, consisting of 51, 12 and 23 cultivars, respectively). Although not statistically significant, considerable differences in %TDM and NUE were observed between the market groups under low and high N conditions. In the rainfed production system, the processing group scored considerably higher than the fresh consumption and general purpose group for both %TDM and NUE especially under low N conditions (Fig. 5a, c). The effect of N levels on %TDM and NUE was also most clear in the rainfed production system. In the irrigation production system, a clear difference was observed only between N levels for NUE (Fig. 5b, d) and not between market groups. Overall, the %TDM value was low; it fell in the range of 10 to almost 20%. This low value of the %TDM might have been associated with the interaction between variety and the climatic conditions (temperature and the length of the growing season) and their interactions. In this study, European potato varieties developed in the European long growing season and photoperiod condition were used and they might not perform well when grown outside the target/selection environment. The climatic condition of the experimental region (Ethiopia) can be characterized as short growing season with high temperature; these climatic conditions might reduce the %TDM content. Especially the irrigation production was conducted in the dry months of the year and in this period the temperature was high which might have led to high respiration. The high respiration rate might shorten the lifespan of the crop resulting in low %TDM content.

Higher tuber yield and NUE values were recorded under irrigation compared with the rainfed production season. This might have been related to the amount and distribution of rain in the rainfed season. The rainfall was heavy and frequent from mid-June to August (growing and bulking period of potato), and this high rainfall favored potato diseases and insect pest occurrence, which might have reduced the tuber yield and NUE. In fact, we applied a fungicide (Redomil MZ 68 WG, Syngenta) for the management of late blight on the foliage before infection or when the disease was in its very early stages. Nevertheless, confounding disease symptoms were observed in addition to late blight that may have reduced the performance of the cultivars. Heavy and frequent rain might also have reduced available N in the rainfed locations compared with the irrigated locations.

Phenotypic association among traits may be good evidence that the two traits are functionally and genetically linked with each other or not. The Pearson correlation coefficients ($r$) between various physiological and agronomic traits under low N and high N conditions are presented in Table 7. NUE showed strong positive correlations with Vmax, AP3, AUC, TNPP, TYPP and %TDM under both N levels indicating the
Table 6  Summary statistics of the association mapping panel for various agronomic and physiological traits (combined over locations and production systems) under low N (LN) and high N (HN) conditions

| Traits | Treatment | Mean | Range | Variance component |
|--------|-----------|------|-------|-------------------|
|        |           | Minimum | Maximum | σ²e | σ²g | σ²ge | H² |
|        | LN        | 24.0 | 20.0 | 28.0 | 9.6 | 1.01 | 2.08 | 0.37 |
|        | HN        | 25.0 | 22.0 | 29.0 | 18.9 | 0.06 | 1.01 | 0.02 |
| PH     | LN        | 31.0 | 25.0 | 39.0 | 16.3 | 5.7 | 14.3 | 0.50 |
|        | HN        | 38.0 | 29.0 | 47.0 | 19.9 | 8.5 | 16.2 | 0.56 |
| SNPP   | LN        | 3.0  | 2.0  | 5.0  | 0.5  | 0.2  | 0.2  | 0.55 |
|        | HN        | 3.0  | 2.0  | 5.0  | 0.4  | 0.2  | 0.3  | 0.54 |
| LCC    | LN        | 44.5 | 38.8 | 49.4 | 15.4 | 1.6  | 3.6  | 0.36 |
|        | HN        | 47.3 | 42.7 | 52.8 | 12.8 | 1.2  | 7.8  | 0.25 |
| UCC    | LN        | 42.4 | 37.1 | 47.1 | 10.0 | 0.6  | 3.6  | 0.23 |
|        | HN        | 46.0 | 42.8 | 51.2 | 8.2  | 0.8  | 7.3  | 0.23 |
| DTM    | LN        | 74.0 | 63.0 | 81.0 | 69.8 | 0.7  | 7.9  | 0.06 |
|        | HN        | 73.0 | 65.0 | 80.0 | 65.3 | 1.2  | 14.4 | 0.09 |
| tm1    | LN        | 23.4 | 19.0 | 25.4 | 8.6  | 0.05 | -0.6 | 0.05 |
|        | HN        | 22.4 | 19.1 | 25.0 | 12.8 | 0.2  | -1.4 | 0.16 |
| t1     | LN        | 30.4 | 27.5 | 32.1 | 7.9  | 0.07 | -0.3 | 0.07 |
|        | HN        | 30.2 | 27.8 | 33.8 | 10.1 | 0.07 | 0.3  | 0.05 |
| t2     | LN        | 36.5 | 34.5 | 38.9 | 9.9  | 0.03 | 0.7  | 0.02 |
|        | HN        | 36.2 | 33.2 | 39.1 | 8.2  | 0.01 | 2.3  | 0.01 |
| Vmax   | LN        | 51.7 | 47.7 | 54.7 | 5.2  | 0.5  | 1.5  | 0.31 |
|        | HN        | 50.5 | 48.1 | 52.5 | 5.9  | 0.2  | 0.6  | 0.21 |
| t2-t1  | LN        | 6.1  | 3.4  | 8.5  | 10.2 | 0.01 | 1.0  | 0.00 |
|        | HN        | 14.3 | 10.3 | 18.3 | 12.9 | 0.4  | 2.8  | 0.00 |
| AP1    | LN        | 45.4 | 36.5 | 58.6 | 90.5 | 7.06 | 31.4 | 0.27 |
|        | HN        | 61.5 | 42.9 | 76.7 | 113.7| 17.7 | 51.0 | 0.40 |
| AP2    | LN        | 255.8| 145.3| 406.8| 21072.0| 264.6| 5230.0| 0.06 |
|        | HN        | 349.3| 208.4| 639.1| 37189.0| 1919.8| 8780.0| 0.22 |
| AP3    | LN        | 480.2| 315.5| 706.2| 30944.0| 1259.4| 2315.0| 0.22 |
|        | HN        | 607.3| 370.9| 806.6| 37029.0| 1248.9| 13926.5| 0.13 |
| AUC    | LN        | 1139.6| 883.4| 1575.6| 58737.0| 6313.9| 32060.0| 0.29 |
|        | HN        | 1608.8| 974.8| 2174.6| 113083.0| 217184.1| 3200749.5| 0.06 |
| TNPP   | LN        | 5.4  | 3.8  | 7.7  | 1.5  | 0.3  | 1.2  | 0.39 |
|        | HN        | 7.0  | 5.4  | 10.2 | 1.5  | 0.5  | 0.6  | 0.43 |
| ATW    | LN        | 74.8 | 44.8 | 103.9| 314.9| 57.3 | 201.2| 0.39 |
|        | HN        | 81.6 | 55.0 | 106.4| 444.1| 49.2 | 131.6| 0.36 |
| TYPP   | LN        | 380.2| 269.5| 558.3| 10165.0| 673.0| 3193.0| 0.25 |
|        | HN        | 540.4| 353.0| 771.4| 28994.0| 1345.4| 5128.0| 0.22 |
| TDM%   | LN        | 13.2 | 9.6  | 16.9 | 13.9 | 0.6  | 1.6  | 0.23 |
potential of these traits for indirect selection for NUE under low and high N level conditions. Most traits considered in this study showed medium to high positive correlations both at low and high N conditions. The associations of the canopy cover traits, NUE and tuber yield with maturity were low. The possible reason for the weak association of maturity with these important traits might have been the effect of environment. Under Ethiopian growth conditions, the varieties matured on average within 74 days and under these different conditions, it might have been difficult to show its clear relationship with yield, NUE and canopy dynamics.

Table 6 (continued)

| Traits | Treatment | Mean Maximum | Range | Variance component |
|--------|-----------|--------------|-------|--------------------|
|        |           | Minimum      |       | $\sigma^2_e$ $\sigma^2_g$ $\sigma^2_{ge}$ $H^2$ |
| HN     | LN        | 12.1 9.6 16.7 | 10.4 0.6 0.7 0.29 |
| NUE    | LN        | 59.4 36.0 98.4 | 816.4 19.3 -56.0 0.18 |
| HN     |           | 27.0 16.6 38.9 | 196.2 3.1 -14.2 0.13 |

$DTE$ days to emergence, $PH$ plant height (in cm), $SNPP$ stem number per plant, $LCC$ lower leaf chlorophyll content (SPAD unit), $UCC$ upper leaf chlorophyll content (SPAD unit), $DTM$ days to maturity (in days), $tm1$ inflection point in the build-up phase of the growth curve (in days), $t1$ time when the canopy cover reaches its maximum growth (in days), $t2$ time of onset of canopy declining (in days), $te$ time when canopy cover reaches zero (in days), $t2-t1$ duration of maximum canopy (in days), $te-t2$ duration of senescing of the canopy (in days), $Vmax$ the maximum canopy cover (in %), $AP1$ area for growth phase one (emergence until $t1$) (% td), $AP2$ area for growth phase two ($t1$ until $t2$) (% td), $AP3$ area for growth phase three ($t2$ until $te$)(% td), $AUC$ area under the curve for the entire crop growth cycle (in % td), $TNPP$ tuber number per plant, $ATW$ average tuber weight (g), $TYPP$ tuber yield per plant (g/plant), $%TDM$ tuber dry matter content (%), $NUE$ nitrogen use efficiency (kg/kg)

Fig. 5 a-d Box plots of selected traits of cultivars separated in market niche groups under different N levels in rain-fed and irrigation production systems. LNFC—low nitrogen fresh consumption, LNG—low nitrogen general purpose, LNP—low nitrogen processing, HNFC—high nitrogen fresh consumption, HNG—high nitrogen general purpose, HNP—high nitrogen processing type, $%TDM$—tuber dry matter percentage, $NUE$—nitrogen use efficiency
Table 7. Correlation coefficients of the four-environment combined agronomic and physiological traits of the association mapping panel under high N (above diagonal) and low N (below diagonal), with the diagonal indicating the correlation coefficient value of a trait between low and high N For the coding of the variables see Table 6.

|           | High N | Low N | DTE | SNPP | UCC | tm1 | tl | t2 | te | Vmax | t2-t1 | te-t2 | AP2 | AP3 | AUC | DTM | TYP | TNPP | TDM % | NUE |
|-----------|--------|-------|-----|------|-----|-----|----|----|----|------|-------|-------|-----|-----|-----|-----|-----|------|-------|-----|
| DTE       | 0.35   | -0.26 | 0.23| 0.09 | -0.13| -0.14| -0.14| -0.11| 0.13| -0.02| 0.03  | 0.12  | 0.13 | 0.15| -0.17| 0.12| -0.05| 0.02 | 0.1 |
| SNPP      | 0.41   | 0.74  | -0.23| -0.14| 0.02  | 0.07 | 0.06 | 0.1  | 0.05| -0.01| 0.08  | 0.03  | 0.04 | 0.08| 0.03 | 0.41| 0.07 | 0.03 |
| UCC       | -0.15  | 0.01  | 0.46 | 0.23 | -0.01| 0.11 | 0.09 | -0.16| 0.12| -0.02| -0.02| -0.09 | -0.1 | 0.28 | 0.15| -0.23| -0.08| 0.17 |
| tm1       | -0.23  | 0.09  | 0.08 | 0.00 | 0.55 | 0.21 | 0.08 | 0.21 | -0.31| -0.11| -0.11| 0.12  | 0.09 | 0.2  | 0.11 | -0.06| 0.01 | 0.14 |
| tl        | 0.3    | 0.27  | 0.02 | 0.46 | 0.14 | 0.47 | 0.21 | 0.06 | -0.48| -0.22| -0.33| -0.05 | 0.04 | 0.24| -0.18| -0.01| 0.01 | 0.15 |
| t2        | 0.06   | -0.05 | 0.04 | 0.36 | 0.39 | 0.36 | -0.01| 0.25 | 0.55 | -0.76| 0.54  | -0.32 | 0.3  | 0.19 | 0.04 | 0.22 | 0.21 | 0.12 |
| te        | -0.36  | 0.23  | 0.11 | 0.62 | 0.42 | 0.06 | 0.49 | 0.35 | -0.2 | 0.66 | -0.08 | 0.7  | 0.37 | 0.44| 0.31 | 0.14 | 0.03 | 0.23 |
| Vmax      | 0.18   | -0.02 | -0.22| 0.22 | 0.22 | 0.26 | 0.15 | 0.59 | 0.19 | 0.05 | 0.6   | 0.72  | 0.93 | 0.23| 0.54 | 0.53 | 0.27 | 0.53 |
| t2-t1     | 0.33   | -0.29 | 0.01 | -0.09| 0.55 | 0.55 | -0.33| 0.03 | 0.32 | -0.54| 0.85  | -0.27 | 0.27 | -0.04| 0.22 | 0.23 | 0.21 | 0.26 |
| te-t2     | -0.33  | 0.21  | 0.06 | 0.26 | 0.08 | -0.6 | 0.76 | 0.04 | -0.62| -0.44| -0.46 | 0.07  | 0.01 | 0.15| 0.17 | -0.07| -0.14| 0.06 |
| AP2       | 0.39   | -0.28 | -0.13| 0.1 | 0.3  | 0.57 | 0.15 | 0.55 | 0.79 | -0.49| 0.57  | 0.08  | 0.65 | 0.03 | 0.37 | 0.43 | 0.34 | 0.44 |
| AP3       | -0.09  | 0.12  | -0.07| 0.32 | 0.22 | -0.19| 0.61 | 0.74 | -0.38| 0.61 | 0.08  | 0.5   | 0.68 | 0.26 | 0.48 | 0.28 | 0.06 | 0.39 |
| AUC       | 0.16   | -0.01 | -0.14| 0.15 | 0.21 | 0.26 | 0.24 | 0.96 | 0.05 | 0.02 | 0.57  | 0.77  | 0.22 | 0.2  | 0.46 | 0.48 | 0.3  | 0.49 |
| DTM       | -0.52  | 0.08  | 0.22 | 0.37 | 0.32 | 0.09 | 0.5  | 0.15 | -0.21| 0.34 | -0.09 | 0.35  | 0.2  | 0.16 | 0.16 | 0.05 | -0.06| 0.05 |
| TYP       | -0.09  | -0.05 | 0.12 | 0.11 | 0.29 | 0.26 | 0.6  | 0.16 | 0.02 | 0.39 | 0.52  | 0.62  | 0.18 | 0.53 | 0.38 | -0.09| 0.78 |
| TNPP      | -0.14  | 0.38  | 0.2 | 0.21 | 0.08 | 0.25 | 0.3  | -0.12| 0.15 | 0.03 | 0.34  | 0.27 | 0.11 | 0.36 | 0.36 | 0.29 | 0.46 |
| TDM %     | -0.1  | 0.15  | -0.16| 0.09 | 0.09 | -0.01 | 0.1 | 0.32 | -0.09| 0.09 | 0.14  | 0.28  | 0.03 | 0.07 | -0.03 | 0.18 | 0.52 | 0.48 |
| NUE       | -0.05  | 0.11  | 0.07 | 0.2 | 0.21 | 0.2  | 0.31 | 0.57 | -0.01| 0.12 | 0.27  | 0.54  | 0.58 | 0.25 | 0.67 | 0.39 | 0.64 | 0.32 |

Association Mapping

Association mapping was performed with 12,519 polymorphic SNP markers, using a Q + K matrix to correct for population structure and kinship in the association panel. We defined the marker-trait associations (MTA) that were within a 10Mb region as a single QTL, and only reported the peak marker in this region. QTLS above the calculated threshold of $p \leq 0.05$ (-log(10)$P$ value of 4.5) for NUE and NUE-related agronomic and physiological traits measured in 15 environments including the over-environment combined data, with allele frequencies above 5%, are presented in Supplementary Table 5. The significant MTAs in these QTLS had –log10(P) values ranging from 4.52 to 7.28. In total, 77 QTLS were detected for 18 measured or calculated phenotypic traits in 8 low and high N single environments, and production season, low N and high N combined environments (Supplementary Table 5). Of the identified QTL regions, 18 harbored QTLS for two or more traits (Table 8).

QTLS for NUE and other agronomic and physiological traits that had a strong positive correlation with NUE were detected on chromosomes, III, V and VI under various environments. On chromosome III, QTLS for NUE and AUC co-localized at the same genomic region in low N environments, while QTLS for $t_2$, $t_2$-$t_1$, DTM and %TDM under different environments were clustered on another region of the same chromosome. SNPP, tm1, t2, t2-$t_1$, te, Vmax, AUC, %TDM and NUE had QTLS on chromosome V, with QTLS for Vmax, AUC and %TDM co-localizing in the same region with NUE between 351,772 and 9,824,216bp. A region between 52,929,083
and 58,844,975bp on chromosome VI harbored QTLs for NUE and other NUE-related traits such as TNPP, UCC and AP2.

AUC and DTM shared an association with the same marker (PotVar0010985) on chromosome IX under high N conditions in the rainfed production season at Injibara. Other QTLs for AUC were detected on chromosomes III, V and IX, and marker PotVar0019456 on chromosome III was associated with AUC in over location and production season combined low N environment and irrigation production season combined low N environment. This QTL can be considered as a low N dependent but location and production season-independent QTL for AUC. QTLs were detected on chromosomes I, IV, XI and XII for TYPP, and particularly QTLs linked with the marker solcap_snp_c2_26796 on chromosome IV and marker PotVar0060022 on chromosome XI were associated with TYPP in two low N condition environments under irrigation production system, indicating that these QTLs were low N as well as irrigation season dependent. QTLs detected for traits t1, Vmax, AP3, DTM and NUE were specific for rain-fed production season, while TYPP and te-t2 were irrigation production season specific QTLs. SNPP, tm1, t2-t1, AP2, TNPP and TYPP had QTLs in more than one environment under both production systems. Constitutive QTLs were detected for the major tuber yield component trait TNPP on chromosome VII with marker solcap_snp_c2_26796 and for SNPP on chromosome X with marker PotVar0116800 in different environments.

We detected more than one QTL for most traits in several environments, but environment-specific or environment-excluding QTLs were also identified, suggesting presence of QTL × environment interaction. Only 8% of these QTLs were detected in

| Chromosome | Traits | Chromosome region (bp) |
|------------|--------|------------------------|
| I          | t2 & TYPP | 51,293,720–58,197,448 |
|            | SNPP, DTE & AP2 | 75,179,906–82,029,061 |
| II         | TNPP, t1, t2-t1 & SNPP | 50,890,271–55,659,310 |
| III        | NUE & AUC | 2,235,688–2,742,393 |
|            | TDM%, t2-t1, DTM & t2 | 50,890,271–55,659,310 |
| IV         | DTM, t2-t1 & AP3 | 66,147,280–67,807,068 |
|            | TYPP & te | 641,790–9,524,541 |
| V          | tm1, NUE, SNPP, TDM% & t2-t1 | 2,964,094–12,658,442 |
|            | t2 & te | 50,863,328–51,682,609 |
|            | Vmax & AUC | 351,772–1,413,732 |
| VI         | NUE, TNPP, UCC & AP2 | 52,929,083–58,844,975 |
| VII        | te-t2, AP2, TNPP & t2-t1 | 44,288,221–50,155,639 |
| VIII       | SNPP & TDM% | 53,835,553–56,624,935 |
| IX         | te, UCC, AUC & DTM | 52,407,969–57,422,879 |
|            | AP2 & tm1 | 48,735,918–49,977,704 |
| X          | SNPP | 1,910,636 |
| XI         | TNPP & tm1 | 40,967,802–44,430,446 |
| XII        | TNPP & TYPP | 59,793,350–59,957,211 |
two or more environments and the remaining 92% were environment-specific. Of the total identified QTLs in both single and combined low and high N environments, 49% were detected in high N level environments, 46% in low N level environments and 5% in combined environments. From the detected QTLs, 38 (49%) were detected only in rain-fed and 21 (27%) only in irrigation production systems. Overall, most QTLs identified in this study were environment-dependent indicating presence of QTL-by-environment interaction in our association panel.

To detect MTAs and QTLs, various gene models were used in separate association analyses. In this study, a simplex-dominant genetic model, duplex-dominant genetic model, additive and general genetic models were used to identify MTAs, and the majorities (75%) of the MTAs were identified using dominant genetic models. All MTAs identified for NUE, AUC and t1 were using dominant genetic models, while other traits had MTAs detected by general, dominant and additive genetic models. The quantile-quantile (QQ) plots (Fig. 3b, d, f and h) demonstrate that the Q + K mixed model allowed us to reduce the false marker trait associations, as quantified by the linear regression coefficient of the observed vs expected –log(10)P values.

**Discussion**

**Phenotypic Variation**

NUE is a complex physiological process determined in potato by the efficient uptake, accumulation and partitioning of nitrogen and assimilates to facilitate production of tubers. Uncovering the mechanisms underlying such a complex trait is not straightforward due to the complexity of the adaptive response of the crops to changes in available N. In this study, we present the phenotypic variation of potato cultivars for NUE and genotypic variation (genetic analysis) of NUE and, linking traits that contribute to NUE under low and high N conditions to specific genomic regions in potato. Eighty-eight genotypes were used for the association mapping study. In fact, more genotypes should have been included in this study; however, the challenge to manage more genotypes in the designed experiment at field condition, lack of uniform size seed tubers and lack of marker data for some of the newly released potato cultivars included in the field experiment forced us to limit the number of genotypes included in this study. However, the materials are also studied extensively by other authors for different diversity studies. For example, D’hoop et al. (2008) used these materials in association mapping for quality traits; Ospina et al. (2021) used the materials for NUE efficiency under Netherlands conditions. Both results revealed that there was no clear structure in the panel, but the genotypes were diverse suggesting that the panel was suitable for association mapping studies.

Analysis of genotype-by-environment interaction for a key trait is an important topic of crop variety trial data analysis. To assess mega-environments, and test-environments in a genotype-by-environment analysis, European commercial potato cultivars were evaluated for NUE in eight test environments (E1 to E8) in northwestern Ethiopia. The effect of environmental conditions on NUE and related traits like canopy cover and maturity of potato was highly significant. This may be associated with limited adaptation to the experimental conditions, since the Dutch cultivars were selected for

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Western-European, long day conditions. Haverkort (1990) suggested that potato varieties adapted to long day conditions may mature earlier and senesce between 60 and 70 days after emergence when grown around the equator. This physiological change is likely related to environmental factors like photoperiod and temperature. According to Ewing and Struik (1992), photoperiod and temperature are the major environmental factors that influence the growth of potato. Reduction in vegetative growth, early tuberization and senescence are the response of potato under short day condition (Maris 1964; Haverkort 1990; Van Dam et al. 1996).

The results of the field trials demonstrated the impact of environment, and in particular production season on the NUE of potato cultivars. Large contributions of the environment that influenced NUE of genotypes were reported in a number of studies (Bertin and Gallais 2000; Dawson et al. 2008; Agostini et al. 2010; Liu et al. 2012). Significant G×E interaction can result in different ranking of potato cultivars and complicate selection, because measured performance in one environment fails to predict performance in another environment (Baker 1988). To gain more insight in the influence of environment on potato cultivar performance and to increase selection efficiency, a GGE biplot analysis was done to evaluate test environments and cultivars. In the GGE biplot analysis, usually the first two principal components (PC 1 and PC2) are needed explain the data structure, even though other PCs that have no significant contribution to explain the biplot may be detected (Gauch and Zobel 1997; Yan et al. 2000). In our study, the first two PCs of the biplot indeed accounted for more than 50% of the GGE, demonstrating that in the GGE biplot model the first two PCs can be used to explain interpretable patterns of the GGE.

Figure 2b shows that radiates of the biplot divided the plot into seven sectors, with four environments all appearing in one sector (left of the biplot), and the remaining four appearing in the right side of the biplot. These two sectors had different high NUE vertex genotypes indicating the presence of crossover G×E interaction, and suggesting the test environments should be divided into mega environments (Yan et al. 2007). Understanding and identifying mega environments can result in increased heritability through evaluations in relatively well-defined and predictable target environments (Abdalla et al. 1996). This targets the most promising genotypes for a target trait and improves the efficiency of breeding programs. Our results suggest that there are at least two potato mega environments for potato NUE evaluation in northwestern Ethiopia, coinciding with the rainfed and irrigation production systems (Fig. 2b).

The relevance of these two mega environments is supported by the fact that environments did not cluster based on location: Environments E3, 4, 5 and 6 are all in Injibara, but these were divided over the two mega-environments based on production system, demonstrating that season was the main contributor for the G×E interaction and the driver for the formation of the two mega environments. The highest performance of the varieties was recorded at E7 and E8 (at Koga low N and high N condition) respectively, under irrigation conditions. This high environmental variance compared with genotypic variance particularly in the irrigation production system may be attributed to irrigation management. As we conducted the field experiment using furrow irrigation, seepage and other factors might have enhanced within and between field variations, and thus increased the environmental variance in the irrigation experiments. Previously, CIP-sourced Ethiopian clones and local cultivars were evaluated under rainfed and irrigation production systems in northwestern Ethiopia, but stable
cultivars for both rainfed production system and irrigation production system were not found, indicating that an independent selection program is required for each production system (Yigzaw et al. 2008). However, to reach a final conclusion, and to recommend different selection programs for each production system, this result needs to be confirmed in trials over more years, and possibly more locations.

Understanding and selecting suitable test environments is one of the most important factors for the success of any plant breeding program (Yan et al. 2011). For selecting a test environment, the discriminating ability and representativeness of the target environment should be taken into account (Yan 2002; Xu et al. 2014). In the present study, the two test environments represented by high N level at both Debre-Tabor and Injibara under rainfed production condition had good discriminating ability and representativeness of the rainfed mega environment, suggesting these test environments may be good test environments for both low and high N cultivation conditions. In the irrigation mega-environment, however, the low N test environments were more discriminating and representative, which may suggest that under irrigation production systems, evaluation and selection of cultivars for NUE improvement may be conducted under low N conditions. According to Murphy et al. (2007), the most efficient way to improve crop’s yield under low input conditions is indeed to select varieties under low input or stress conditions. In the irrigation mega environment, the low and high N environments at Injibara (E5 and E6) were strongly associated with each other. The analysis of variance at this location also showed non-significant genotype by N level interaction, which means differences between genotypes are consistent from low N to high N environment. This suggests no significant shift in rank order of genotypes with respect to NUE occurred between N levels for these trials at Injibara. The absence of a significant N effect may be attributed to the strong acidic nature of the soil in Injibara area, as low pH may affect the availability of N also under the high N conditions. Atlin and Frey (1989) found no genotype by N level interaction in oat yield, suggesting the possibility of indirect selection (i.e. selection at low N or high N for both high and low N conditions). However, this may be dependent on the genotypes used, and conclusions should be drawn with care.

The environment-combined variance component analysis showed low genotypic variance (\( \sigma^2 g \)) compared with estimates of genotype-by-environment interaction variance (\( \sigma^2 ge \)) and environmental variance (\( \sigma^2 e \)) for all traits, indicating presence of large differences between environments. In particular, the genotype-by-location interaction, which includes production season, had larger contributions to the total genotype-by-environment interaction than the genotype-by-N level interaction, indicating a significant effect of test locations as well as production seasons on the performance of potato cultivars. In most potato-growing countries, potato cultivars grown in specific areas are selected according to the environmental conditions prevailing in these countries. Lisinska and Leszczyński (1989) reported that tuber yield and quality traits of potato were generally linked to the prevalent climatic conditions of a given area. In our study, the large contribution of genotype-by-location interaction to total genotype-by-environment interaction could at least partly be attributed to differences in production season (rain-fed vs irrigated), and these may be the predominant non-genetic factors affecting growth, yield and NUE of potato. This high environmental variance compared to genotypic variance particularly in the irrigation production system may be attributed to irrigation management. As we conducted the field experiment using furrow irrigation
method, seepage and other factors may enhance within field variation, and thus increase the environmental variance in the irrigation experiments.

NUE and most-NUE-related traits had high genotypic variance and heritability estimates under rain-fed production season conditions, whereas the estimates were low for most traits under irrigation (Supplementary Table 4). The $H^2$ estimate differences between locations and N levels within each production season were small for most traits, while it was large between production seasons (rain-fed vs irrigation) in the locations combined data for most agronomic and physiological traits (Supplementary Table 4). This further indicates, production season contributing more to the total genotype-by-environment interaction than locations and N levels. Thus, our study suggests that the target breeding environment should be divided into sub-target environments (mega-environments) based on production seasons, so as to increase the heritability and selection efficiency of potato for NUE as indicated above in genotype-by-environment interaction analysis.

The %TDM was low in both the rainfed and irrigation production season. This low %TDM might have been attributed to the length of the growing season, temperature and the interaction of the potato varieties with these environmental factors. The climatic conditions of Ethiopia can be characterized as short growing season and short photoperiod, with high temperature which may lead to low %TDM; particularly during the irrigation production season, the temperature was high since it was conducted in the dry months of the year. Above and beyond the environment effects, the genotype effect was also highly significant on the NUE performance of the cultivars. Among the market groups in our potato genotype set, the processing type group had higher values of %TDM and NUE compared to the other two groups especially under rainfed conditions. Bártá and Bártová (2008) determined extractable protein in European processing and table potato cultivars and reported that tuber dry matter percentage was significantly higher in processing potato cultivars than table potato cultivars, which is in line with our results under rainfed conditions. Remarkably, this difference was not observed under irrigated conditions. Whether this difference is related to a season influence, remains to be established.

**Genetic Variation for NUE in Potato**

The phenotypic data analysis showed that there is genetic variation in the panel population for traits related to NUE, indicating that the population may be a suitable panel for detection of QTLs for these traits provided that the population structure and linkage disequilibrium (LD) are acceptable. Population structure (unaccounted sub-populations in the genotype set) induces LD between unlinked loci (Pritchard et al. 2000). Consequently, some marker-trait associations that are statistically associated to the analyzed trait may not be genetically associated with the phenotypic variation (Mezmouk et al. 2011). We used Q and K matrixes to correct for the population structure and kinship, moreover linkage disequilibrium (LD) and population structure were calculated previously for a larger genotype set by d’Hoop et al. (2010) that included the 70 cultivars used for this study. Both results revealed that there was no clear structure in the panel, suggesting that it is suitable for association mapping studies. In the identification of significant MTAs, we tried to reduce false positives. As shown in the quantile-quantile plot, inflation of the P-value above the linear
regression line was very low, which is an indication that the models successfully control the population structure (Fig. 6). Moreover, rare alleles were removed and the

![Manhattan plots and QQ plots for several traits using different gene models.](image)

**Fig. 6** Manhattan plots and QQ plots for several traits using different gene models. a) Manhattan plot of the simplex dominant model for area under the canopy curve for maximum canopy cover phase (AP2). The $-\log_{10} P$-values from the genome wide scan are plotted against the positions of the 12 chromosomes. The horizontal dotted line indicates the significance threshold ($P\leq0.05$). b) Quantile-quantile (QQ) plot of the simplex dominant model for AP2. c) Manhattan plot of the duplex dominant model for stem number per plant (SNPP). d) Quantile-quantile plot of duplex dominant model for SNPP. e) Manhattan plot of additive model for tuber number per plant (TNPP). f) Quantile-quantile plot of additive model for TNPP. g) Manhattan plot of general model for complete canopy senesced (te). h) Quantile-quantile plot of the general model for te.
MTAs were identified using Bonferroni correction as a threshold test, which is one of the most conservative approaches to avoid spurious positives (Rosyara et al. 2016). All the aforementioned tests make it likely that the identified MTAs are indeed resulting from genetic linkage of the markers to the phenotypic traits.

The cultivars in the association panel were mainly developed for high N input and European long day conditions, and they may lack genetic diversity and optimal alleles for NUE and related traits under low N conditions and short days, which is the most common cultivation environment in Ethiopia. Many authors indeed reported that modern plant breeding may have reduced crop genetic diversity (Borlaug 2007; Babiker et al., 2015; Govindaraj et al. 2015), and this may be especially true for traits that were not selected for. However, in our panel, there was still considerable variation for NUE and related traits under both low and high N conditions. This variation may be attributed to the fact that it includes cultivars released for production over a wide range of years and for different purposes. Based on the frequency of genotypes in the markers scored, the processing cultivars contributed more positive alleles to the identified NUE QTLs under low N level condition, while fresh consumption type cultivars contributed more compared with processing types under high N. Of the 8 cultivars which have more positive allele contribution for the identified QTLs under low N, 5 cultivars were processing type. Under high N, 7 cultivars showed higher contribution of positive alleles of which 4 were fresh consumption type. This suggests that the two groups have N level-specific genetic potential for NUE improvement of potato. QTL mapping using the C×E diploid backcross population conducted in a similar field experimental set-up with this study resulted in the detection of several QTL regions (Baye Getahun et al. 2020b). In this diploid backcross population QTL mapping study, many QTLs were detected on chromosome V, with QTLs controlling NUE and other physiological and morphological traits accumulating at different genomic locations. However, QTLs found with a bi-parental mapping approach often cannot directly lead to identification of candidate genes, mainly due to the often low resolution of bi-parental QTL mapping (Bernardo 2008). Association mapping typically has a higher resolution compared to QTL mapping due to the higher number of recombination and it does not confound the analysis of non-additive gene effect like dominant gene effect. In our association mapping approach under low and high N conditions, we identified QTLs in different regions of the potato genome, including several associations accumulating on chromosome V (Supplementary Table 5).

Markers associated with Vmax and AUC co-located between 351,772 and 1,413,732bp on chromosome V. The SNP marker PotVar0026355 on chromosome V, positioned at 4,335,324bp, associated with NUE in the present study and is 3.83Mb away from the PotSNP573 marker positioned at 507,660bp which was associated with NUE in the C×E QTL mapping study (Baye Getahun et al. 2020b). The marker PotVar0026355 is also about 0.34Mb away from the SSR marker Mando located at 4.67Mb which was associated with days to maturity in the same linkage mapping study and 0.16Mb from the CDF1 gene (PGSC0003DMG400018408) shown to be responsible for early maturity and involved in photoperiod-induced initiation of tuberisation (Kloosterman et al. 2013). Indeed, Tiemens-Hulscher et al. (2012) reported that differences in NUE under high and low N input conditions were shown to be strongly associated with maturity type. Others reported that late maturing potato cultivars recorded higher NUE values than early maturing ones under both high and low N
conditions (Zebarth et al. 2004; Baye Getahun et al. 2020a; Ospina et al. 2021). AUC on chromosome III and TNPP and AP2 on chromosome VI co-located with QTLs for NUE. These two genomic regions may be useful for NUE improvement in potato other than chromosome V, and these are independent of chromosome V. Trait-specific stable QTLs (QTLs for one specific trait observed in more than one environment) were detected for various NUE-related traits in different environments. Stable QTLs for TYPP with peak markers *solcap_snp_c2_26796* on chromosome-IV and marker *PotVar0060022* on chromosome XI were observed at two low N level environments under irrigation production system, indicating the QTLs may be low N as well as irrigation season dependent. This suggests the presence of QTL × environment interaction. However, the difference in overall number of identified QTLs between low and high N level is lower than the QTL number difference between the two production seasons. This indicates that, QTL × N level interaction was lower than the QTL × production season interaction in line with the stronger contribution of production system to the total genotype × environment interaction compared to N levels, as already discussed. Similarly, studies in rapeseed using multi-environment trials that included various locations and growing seasons showed that a large number of QTLs were stable across N levels, while genotype × location interaction was strong and most of the QTLs were specific to a single trial (Bouchet et al. 2016). In bread wheat, the QTL × environment interaction study of Kuchel et al. (2007) reported that a large portion of the G×E interaction could be explained by interaction of the QTLs with climatic factors.

To identify QTLs, various gene models were used in different association studies. Most QTL studies focus on estimating the additive effect of the QTL, assuming absence of interaction among QTLs (Bocianowski and Krajewski 2009; Rovaris et al. 2011). However, epistatic and dominance effects also play a very important role in controlling the expression of quantitative traits (Bocianowski 2013). In this study, we used additive, simplex and duplex dominant, as well as general models; most of the MTAs (including MTAs identified for NUE, AUC, TNPP and TYPP) were detected using dominant genetic models. This indicates that the source of heritable variation for the identified MTAs is mostly due to dominant gene action or due to the interaction of alleles at a single locus, and that dominant gene effects are important in controlling potato NUE and NUE related traits. Gopal (1998) in his study on early generation of potato general combining ability also reported that non-additive gene effects were more important than additive gene effects in determining potato tuber yield and yield components. Previous selection which may have narrowed the genetic base of the studied genotypes may be one of the possible causes for greater non-additive genetic variance effect for various traits (Plaisted et al., 1962). Killick and Malcolmson (1973) in their potato combining ability study reported that specific combining ability (SCA) is more important than general combining ability (GCA) in most traits, suggesting that traits subjected to directional selection would be expected to show little additive variance. Our results endorse this concept as most of the cultivars used in our panel have been subjected to selection for tuber yield and other tuber quality traits under various potato breeding programs.

Combining QTLs that control traits of interest from different genomic regions in a single genetic background is a challenging mission in plant breeding. The use of markers for loci that accumulate several traits may increase the QTL pyramiding
efficiency in marker-assisted selection. In this study, several traits that contribute to NUE mapped to the same regions on the genome and can thus be introduced in a new cultivar together, thus reducing the challenge of collecting QTLs that control traits of interest from different genomic regions.

In conclusion, G×E interaction has been found to be important in NUE of potato in northwestern Ethiopia. The GGE analysis divided the test environments over two mega-environments. The identification of mega-environments in the northwestern Ethiopian potato production region and seasons may have various implications. First, it offers the opportunity for the potato breeders to exploit more targeted adaptation for NUE and related traits to achieve maximum yield and NUE. Second, it enhances the heritability and the efficiency of testing. In general, the mean performance and stability analysis identified genotypes that had high mean performance and stability for NUE in each of the mega environments, and these may be used as parents for future NUE improvement in potato.

Genome-wide association mapping detected both stable and environment specific QTLs for NUE and NUE related traits. NUE-related traits such as DTM, Vmax and AUC had a strong positive correlation with tuber yield and yield component traits. The co-localization of QTLs for these traits with NUE QTLs suggests that these can be used for indirect assessment of some tuber yield and yield component traits. Multi-trait chromosome regions have been identified on chromosomes III, V and VI associated with NUE and NUE-related traits. Markers found in the aforementioned chromosome regions could be used for future improvement of NUE and related traits through marker assisted selection. However, to use the markers detected on chromosome-V efficiently, it would help to be able to separate the trait earliness from the other traits.

In summary, these results demonstrate that the effect of production season was greater than the effect of N levels on NUE and NUE-related traits under our experimental conditions. Still, critical genomic regions associated with NUE that were stable across potato populations identified. The identification of these multi-environment QTLs on NUE and related traits provides an important first step to advance our understanding of the genetic mechanisms regulating the complex processes governing NUE and related traits in potato.

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Declarations

Conflict of Interest The authors declare no competing interests.

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